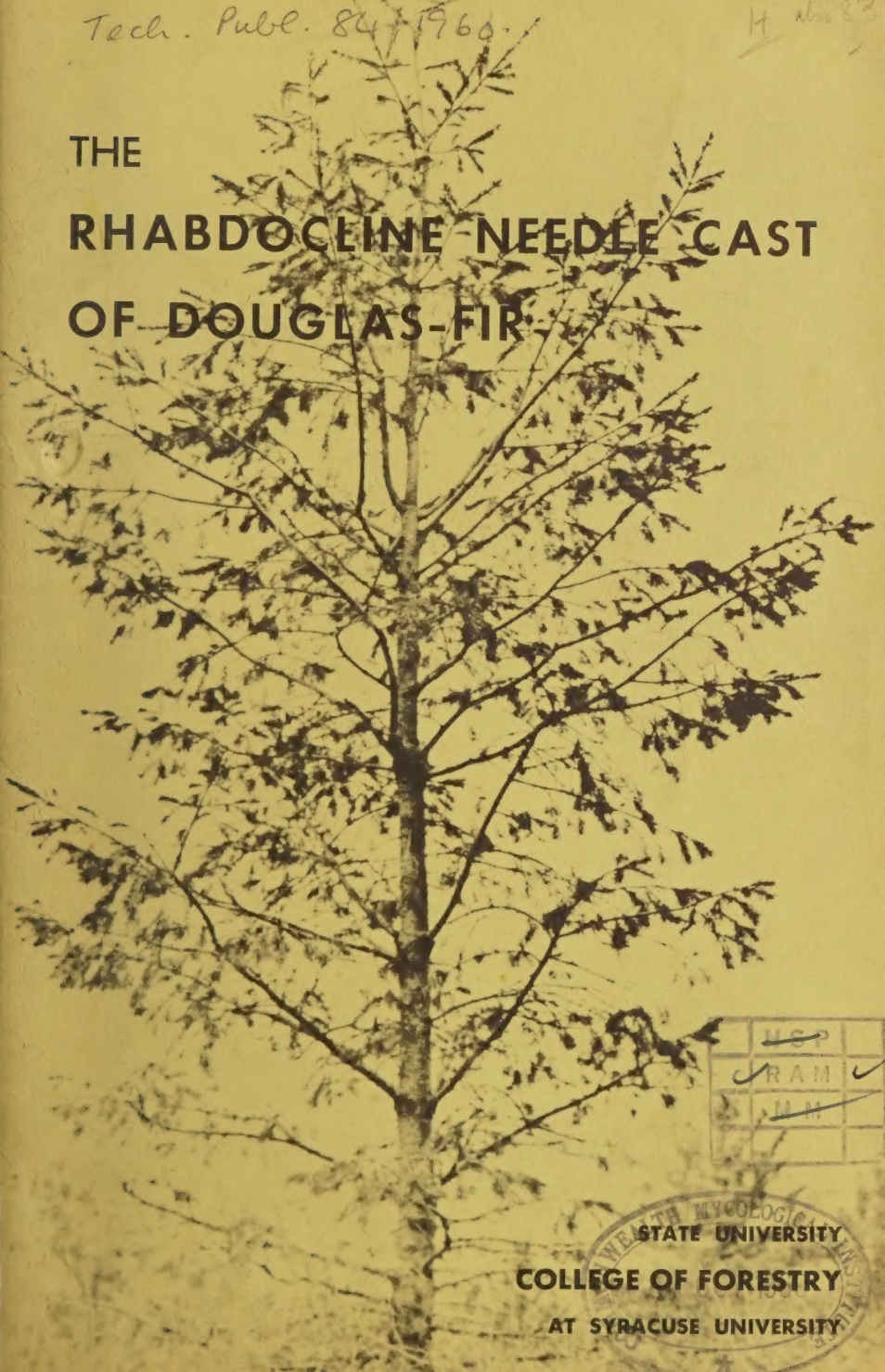
Tech. Publ. 84, 1960.

THE RHABDOCLINE NEEDLE CAST OF DOUGLAS-FIR

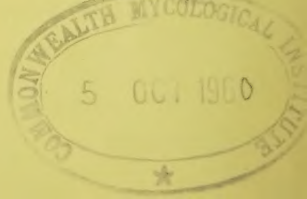


WESTERN MYCOLOGICAL LIBRARY

STATE UNIVERSITY
COLLEGE OF FORESTRY
AT SYRACUSE UNIVERSITY

This publication is available, at a cost of $.80 per copy, from the State University College of Forestry at Syracuse University. Send remittance with order to Department of Forest Extension, State University College of Forestry, Syracuse 10, New York. A list of other publications available through the College may be obtained on request.

Technical Publication No. 84 of the State University College of Forestry at Syracuse University. 1.5M 6/60



THE RHABDOCLINE NEEDLE CAST OF DOUGLAS-FIR

Robert William Brandt

Tech. Publ. N.Y. St. Coll. For.

TECHNICAL PUBLICATION NO. 84 — 1960
STATE UNIVERSITY COLLEGE OF FORESTRY
AT SYRACUSE UNIVERSITY, SYRACUSE 10, NEW YORK

ABOUT THE AUTHOR

ROBERT WILLIAM BRANDT is Plant Pathologist, United States Department of Agriculture, Forest Service, Northeastern Forest Experiment Station, Forest Insect and Disease Laboratory, New Haven, Connecticut.

ACKNOWLEDGMENTS

This paper is based upon a dissertation submitted to the State University College of Forestry at Syracuse University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The research was done under the direction of Dr. Savel B. Silverborg, Associate Professor, to whom the author wishes to express his gratitude. Sincere appreciation is also expressed to Dr. Robert A. Zabel, Chairman of the Department of Forest Botany and Pathology and to Dr. Ray R. Hirt, Professor Emeritus, for their instructive criticisms and valuable advice in planning and presenting the entire study. The author is also indebted to various members of the New York State Conservation Department for use of state areas and for their interest and cooperation.

Certain phases of the studies on control were completed while the author has been an employee of the Forest Insect and Disease Laboratory, Northeastern Forest Experiment Station, United States Department of Agriculture, Forest Service, at New Haven, Connecticut.

CONTENTS

LIST OF ILLUSTRATIONS 5

INTRODUCTION AND HISTORY 7

THE HOST 13

THE PATHOGEN 14

THE DISEASE 16

THE NEEDLE CAST DISEASE IN CENTRAL NEW YORK 21

Development of symptoms 21

Maturation of apothecia 23

Needle casting 24

Sporulation 27

Infection 37

Rate of spread 40

Lammas growth 42

HISTOPATHOLOGICAL STUDIES 45

Normal needle anatomy 45

Diseased needle anatomy 46

CONTROL STUDIES 51

SUMMARY AND CONCLUSIONS 58

LITERATURE CITED 61

ILLUSTRATIONS

Fig. 1. — Douglas-fir infected three years by *Rhabdocline pseudotsugae* 18

Fig. 2. — Cross-section of a stem of Douglas-fir infected five years. 19

Fig. 3. — Lower surface of Douglas-fir needles covered with mature apothecia of the fungus. 23

Fig. 4. — Moth-eaten appearance of twigs when the needles have been infected. 26

Fig. 5. — Cross-section through an apothecium. 27

Fig. 6. — Expulsion of the ascospores from asci. 29

Fig. 7. — Cross-section of asci and paraphyses. 30

Fig. 8. — Partially mature ascospores recently released from the asci. 31

Fig. 9. — Typical early germination stage of the ascospores. 32

Fig. 10. — Typical lateral germ tube shortly after its emergence. 33

Fig. 11. — A germinating ascospore within the apothecium. 34

Fig. 12. — Abnormal enlargement of the hyaline cell of an ascospore. 35

Fig. 13. — Ascospore discharge at the Camden plantation, 1956. 36

Fig. 14. — Ascospore discharge at the Cortland plantation ,1956. 37

Fig. 15. — Ascospores adhering to the surface of a young needle. 38

Fig. 16. — Closeup of an ascospore on a needle prior to germination. 39

Fig. 17. — Globular oily hyphae of *R. pseudotsugae* in the mesophyll cells 47

Fig. 18. — Concentration of hyphae against the endodermal wall. 48

Fig. 19. – Thin intercellular hyphae near the outer margin of the needle. 49

Fig. 20. – Longitudinal view of the mesophyll just below the lower epidermis. 49

Fig. 21. – Cross-section of a needle showing mass of hyphae under the stomata. 50

Fig. 22. – Individual differences in susceptibility to *R. pseudotsugae*. 57

INTRODUCTION AND HISTORY

Rhabdocline pseudotsugae Syd., the cause of a serious needle cast disease, is threatening Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] in plantations in New York and other parts of the Northeast. The fungus infects the newly-developing needles in the spring and early summer and, usually within a year, causes severe damage through excessive needle discoloration and casting. Diseased trees are undesirable as Christmas trees because of sparse, discolored foliage and decreased needle retention. In addition, the growth of severely diseased trees is sharply reduced and death frequently results.

The production of high quality Douglas-fir Christmas trees will require the use of good cultural methods. Foremost among these may be the control of the Rhabdocline needle cast disease. A better understanding of the life history of *R. pseudotsugae,* as it occurs in New York, is needed for successful control. With these thoughts in mind, the general objectives of the present study were: (1) to determine the range of the disease in New York including number of plantations infected and severity of the infections; (2) to determine the life cycle of *R. pseudotsugae* in New York, with particular emphasis on the factors affecting spore release; (3) to investigate the role of late (lammas) shoot development in the spread of the disease; (4) to determine the rate of spread of the disease in plantations in central New York; and (5) to evaluate the effectiveness of various fungicides for the control of the disease.

The Rhabdocline needle cast disease was first observed by Weir in Montana and Idaho in 1911. The first published account (66) was given by him in 1917 at which time he indicated its prevalence, and described the symptoms and causal fungus. He tentatively referred the fungus to the family Stictidaceae of the class Ascomycetes, but did not name it.

An examination of the specimens from Weir's collection by Sydow (58) revealed the fungus to be an undescribed species in the Phacidiaceae, close to the genus *Stegopeziza* of von Hoehnel. Sydow proposed the new genus *Rhabdocline* based on the single species *Rhabdocline pseudotsugae.* From the same needles Sydow described *Rhabdogloeum pseudotsugae* Syd. as a new conidial stage of the fungus. The relationship of *Rhabdogloeum* to *Rhabdocline* has

since been questioned by Van Vloten (63), Ellis and Gill (15), Boyce (5), and Weistaner (68).

Wilson and Wilson (70) first reported the disease in Europe in 1926, and described its symptoms on two varieties of Douglas-fir in Scotland. They traced the development of the fungus in the needles and gave the first summary of the life history of the fungus. The initial infection in Scotland was believed to have originated from Douglas-fir transplant stock imported from the Northwestern United States and Canada. Wind dissemination of ascospores was suggested as the probable source of a second infection in an area eight miles from the first.

Boyce (2) in 1926 reported the epidemic nature of the fungus on Douglas-fir in Scotland as contrasted to its endemic nature in its native habitat. He observed the apparent immunity of the fast-growing green strain of Douglas-fir. A year later, however, Boyce (3) suggested the possibility of fungus adaptation to the green strain.

In his summary of the biology of *R. pseudotsugae,* Day (13) questioned the efficacy of spraying and recommended the use of silvicultural controls.

A review of the Rhabdocline disease situation in England by Wilson (69) outlined chronologically the development of the fungus on the needles and stated that death normally occurred after three to five years, at which time the trees were completely defoliated. He observed the continued apparent resistance of the green strain to the fungus, but questioned its ability to remain immune.

In the United States, Boyce (4) in 1928 found Rhabdocline needle cast in Douglas-fir plantations for the first time in the Northeast (eastern Massachusetts). He theorized that the fungus was introduced on nursery stock, from either the Pacific Northwest or Europe.

Brown (7) made a detailed study of the premature fall of Douglas-fir needles infected with *R. pseudotsugae.* He concluded that lowered water content in infected needles resulted in their early fall.

In 1931, Liese (26) in Germany reported the occurrence of the fungus on the green strain or Douglas-fir. He suggested the probability of long distance spore dispersal as a means of rapid spread of the disease and, as a control measure, recommended cutting and burning all infected branches before apothecial formation in the spring.

Liese (28) summarized the effects of Rhabdocline needle cast on

the various strains of the host and commented on the influence of the disease upon German silvicultural practices for Douglas-fir. A controversy developed on proper control procedures when Geyr (19, 20) opposed both Liese (27) and von Tubeuf (61) on recommended methods for combating the disease. Geyr advocated the location and propagation of resistant strains of Douglas-fir and gradually his views were accepted by the others.

In 1932 Liese (29) compared the susceptibility of the green, blue, and grey strains of Douglas-fir to the needle cast disease. He concluded that the immunity of the green strain was attributable to the late bursting of the buds, after the infecting ascospores of *R. pseudotsugae* had been disseminated. He also reported that lammas branches remained healthy due to their late development. While studying the biology of the fungus, Liese found that twigs with infected needles dried, when cut prior to apothecial maturation (usually April), and ascospores failed to develop. Thus burning was not necessary if severed branches were spread on the ground to facilitate rapid drying. For the practical forester, Liese recommended planting stock from only the fast-growing strains of the coastal or "viridis" varieties of the Douglas-fir

Rohde (43) reported that the age of infected stands in Germany ranged from 13 to 37 years, but gave the period of greatest susceptibility from 15 to 30 years. In the same paper he mentioned one area in northwestern Germany having infected seedlings that were only two years old. Rohde (44) concluded that mixed stands were as subject to the needle cast disease as pure stands, and that the incidence of attack varied greatly with the nature of the season.

Von Tubeuf (61) in 1932 briefly summarized the development of the needle cast disease and compared *R. pseudotsugae* with certain of the Hysteriaceae. He found only the perfect stage of the fungus, and recommended spraying the young needles with a Bordeaux mixture as a preventative measure.

A thesis devoted to *R. pseudotsugae* on Douglas-fir, was published, in part, by Van Vloten (63) in Holland. After unsuccessful attempts to culture the fungus on artificial media, he concluded that *R. pseudotsugae* was truly an obligate parasite. He demonstrated that infection took place through the cuticle on either surface of the needle and that only the chlorenchyma was invaded. He traced the development of the apothecia and searched, without success, for the nature of resistance shown by certain blue and grey strains of the host.

A semipopular account of *R. pseudotsugae* by Wollenweber and Richter (71) was published in 1932. This gave the distribution of the disease in Germany, the symptomatology and life history of the fungus, method of infection, and recommendations for control.

In 1933, Liese (30) publicly repudiated his ideas of direct control consisting of trimming and burning infected branches and accepted Geyr's (19) proposals for locating resistant varieties and developing experimental nurseries. This was prompted by the continued rapid spread of *R. pseudotsugae* in Douglas-fir plantations in Germany.

Rohde (45) continued his efforts to learn the date of introduction of *R. pseudotsugae* into Germany and delimited the distribution of the disease in that country. He determined the primary infection date to be 1922, and in 10 years the fungus had spread to a reported 27 silvicultural districts. He emphasized the need of meteorological studies coupled with data on the rate of spread. Rohde (45) also mentioned the differences in susceptibility of the strains of the host to the needle cast fungus but suggested that such differences were greatly exaggerated. He denounced the practice of cutting diseased trees as a control measure because of the large areas and numbers of trees involved.

In a paper on the mechanics of needle cast in Douglas-fir, Rohde (46) in 1934 rejected Liese's explanation for the lack of infection on the green strain. Even though needles of this strain developed later than those of the grey and blue strains, ascospores were found a month after the bursting of the late developing buds. He concluded that lateness in shoot expansion of the green strain was not entirely responsible for its immunity, but that other factors, such as favorable site and water retention had important effects on the susceptibility of a tree to *R. pseudotsugae*.

Van Vloten (64) traced the long-distance dissemination of the fungus on four-year-old Douglas-fir in Holland and found that they had been infected upon arrival from a forest nursery in northern Germany.

Liese (31) and Rohde (47) both published on the relative susceptibilities of the various strains of the Douglas-fir in 1936. They concluded that the green strain was not infected as commonly as the other strains.

Kanzow (25) found that, for all practical purposes, the green or coastal form of the Douglas-fir could be considered immune. He proposed a scale for such immunity based upon the altitude of the

seed origin. He determined that *R. pseudotsugae* was of minor importance in stands from seed originating below 1,500 meters.

In a review of forest pathology in North America, Peace (40) described *R. pseudotsugae* as consisting of several varieties "varying in the form of their fructifications and spores, and probably also in their pathogenicity."

Ellis (14) reported on new conifer diseases in the southwestern United States in 1939. Among them was *R. pseudotsugae* on Douglas-fir associated with an imperfect stage very similar to *Rhabdogloeum pseudotsugae*. Ellis and Gill (15) published the new *Rhabdogloeum* as *Rhabdogloeum hypophyllum* Ellis & Gill. *R. hypophyllum* was found repeatedly preceding *Rhabdocline pseudotsugae* on Douglas-fir needles in the Southwest. The authors proposed that this fungus was probably the true imperfect stage of *Rhabdocline pseudotsugae* since *Rhabdogloeum pseudotsugae* was very rarely associated with the perfect stage.

In Switzerland, Terrier (59) working with *Adelopus gäumanni* Rohde, the cause of a needle disease of Douglas-fir, found *Rhabdocline pseudotsugae* on the same trees and even the same needles. This was the first published report of the two organisms being found on the same trees and also the first record of *R. pseudotsugae* in Switzerland. The fungus was believed to have been introduced southward from Germany.

In a report on diseases of Douglas-fir in Germany, Zycha (72) compared the damage caused by *R. pseudotsugae* with *Adelopus gäumanni* and found the former more destructive since needles were killed within a year after infection, whereas needles infected with *Adelopus gäumanni* remained on the tree for three years. Once again the resistance of the green form to *R. pseudotsugae* was strongly emphasized, but later Zycha (73) reported the resistance as merely a wide variation in susceptibility.

Meyer (35) in 1951 gave the recent geographical distribution of the disease in Germany, and during the course of his investigations, concluded that differences in resistance were perhaps due to individual tree differences rather than to racial characteristics. He considered Rhabdocline needle cast as the most serious disease of Douglas-fir in Germany. The amount of infection was reduced by two spray applications of copper lime in May, but control was ineffective due to the lack of adhesion of the spray to the lower surfaces of the needles.

R. pseudotsugae was first discovered in Pennsylvania in 1952, and reported by Stambaugh (55) and Stambaugh and Bramble (56). The latter paper reports germination of the ascospores within the apothecia and the apparent relationship between the presence of mature spores and bud opening in the spring.

Murray (39) reported variations in growth and susceptibility to *R. pseudotsugae* for different varieties of Douglas-fir planted in Van Vloten's private estate in Holland. The green forms of the host averaged over 20 feet in height and remained healthy, whereas both the blue and grey forms were infected, the former averaging about 7 feet and the latter 2 feet in height. According to the author, many of the grey Douglas-fir were killed by *R. pseudotsugae*.

Meyer (36) in 1954 studied the degree of susceptibility to needle cast by Douglas-fir in Germany on trees of different origins from the Pacific Northwest. He found the green forms, from Washington, Oregon, and British Columbia, to be resistant, the blue forms (all from Colorado) to be susceptible, and the grey forms to vary considerably in susceptibility according to their origin.

In a study of the disease in Montana, Weistaner (68) in 1955 reported the presence of both imperfect stages, *Rhabdogloeum pseudotsugae* and *R. hypophyllum* on needles prior to the formation of *Rhabdocline pseudotsugae*. The exact relationship of these two fungi to *R. pseudotsugae* was not conclusively determined.

Subsequent reference to *R. pseudotsugae* in the literature relates directly, either to further outbreaks of the disease in countries already noted here, or to the establishment of quarantines for preventing importation of Douglas-fir growing stock. Such reports have no great bearing on the history of the disease and are omitted.

THE HOST

The scientific name of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] honors the Scotch physician and naturalist Sir Archibald Menzies (1754-1842), who discovered the tree in 1791 at Nootka Sound on Vancouver Island, British Columbia (32). In the "Check List of Native and Naturalized Trees of the United States," the United States Forest Service (32) recognizes two varieties of Douglas-fir: (1) *Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco, the typical green or coast variety, known in the European literature as variety "viridis" and (2) *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco, which includes not only the blue or "glauca" variety, but also the grey "caesia" variety of Europe. The latter two are also commonly known as Rocky Mountain and Intermountain Douglas-fir, respectively.

Douglas-fir comprises approximately 60 per cent of the standing timber of our western forests, and furnishes about one-fifth of the total annual cut of timber in America (22). The natural range of the tree is the western United States and British Columbia. The hardy Rocky Mountain form has been grown successfully in the Northeast for Christmas trees.

As first suggested by Wilson and Wilson (70) the habit of Rhabdocline attack on the Douglas-fir in Europe seems to indicate that the viridis, glauca, and caesia varieties are actually distinct strains or races. The three strains of the Douglas-fir in Europe show marked differences in growth rate and susceptibility to various diseases (2, 13, 70). The effects of *Rhabdocline pseudotsugae* in reducing height growth of the grey and blue strains when contrasted to immune green strains on Van Vloten's estate in Holland were reported by Murray (39). The silvicultural differences of the green and blue forms were studied by Frothingham (17) for western United States. He concluded that the coastal green Douglas-fir was faster growing and of better form than the blue Rocky Mountain strain.

Although racial resistance to the disease is not known to be present in the United States, certain individuals show marked resistance to *R. pseudotsugae*. Two adjacent trees of the same age in a plantation may differ in resistance from complete susceptibility to complete or apparently complete immunity (5).

THE PATHOGEN

Little information has been published on *Rhabdocline pseudotsugae* in the United States and Canada since Weir's original description (66). The taxonomy of the perfect stage was completed with Sydow's (58) establishment of the genus *Rhabdocline* based on the single species *R. pseudotsugae*. The fungus was placed by Sydow in the family Phacidiaceae of the order Phacidiales. A description of the genus *Rhabdocline* is as follows:

> Fructifications elongated, subepidermal, without stroma, breaking through the longitudinally splitting epidermis, with convex, brown fruit disk and hyaline small-celled lower layer. Asci clavate, 8-spored. Spores long-ellipsoid, one-celled, hyaline, embedded between simple or branched, coalesced paraphyses.

Rhabdocline pseudotsugae is described as:

> Fructifications on the under side of the needles, scattered or in longitudinal rows; more or less aggregated and running together at the ends. Reddish-brown flecks more or less visible on the upper needle surfaces. Fructifications of different lengths, up to 3 mm. long, about 300μ wide, and 200μ high; appearing subepidermal, the epidermis at first rather strongly distending in the form of pustules but the red-brown hymenium soon breaking out through a long but not prominent slit on the side or top of the epidermal cover, hence resembling an hysterothecium. This hysterothecium having a basal layer 50-70μ or occasionally up to 200μ thick, made up of hyaline, coalesced hyphae of 1.5-2μ diameter and underlaid by a loose hyaline plectenchyma tissue of richly ramifying septate hyphae. Underlying hyphae dissolving away while the remainder grow closer with the epidermal inner wall in a more or less distinct thread-cell hymenium continuing to the upper surface of the fructification. No fungal tissue develops above the hymenium. Asci standing parallel, clublike or nearly oblong clublike, 18-20μ x 120-130μ, broadly rounded above, but gradually attenuated below, 8-spored, sitting embedded among coalesced, thready, forked or simple paraphyses. Ascospores one to two-rowed, cylindrical or oblong-cylindrical, broadly rounded at both ends and often somewhat constricted in the middle, 6-9μ

x 15-20μ. Not constricted when mature. One-celled, hyaline with fine nuclear plasma, rarely with one to two large oil globules when young. Later two-celled with one cell becoming dark; the other remaining hyaline.

The characteristics of *Rhabdocline* still bring it within the family Phacidiaceae and order Phacidiales as interpreted by Clements and Shear (12).

Although the imperfect fungi, *Rhabdogloeum pseudotsugae* and *R. hypophyllum* have been reported commonly associated with *Rhabdocline pseudotsugae* (15, 58, 68, 70) they were not found in Central New York.

THE DISEASE

The first visible evidences of the Rhabdocline needle cast disease appear in the autumn or early winter as minute yellow spots on the upper and lower surfaces of the current year's needles. These spots are irregularly scattered over the surfaces of the needle but occur more abundantly near the apex. During the winter months the spots become larger and darker through an increase in number of necrotic cells in the lesion. By the following spring many of the spots coalesce and become deep red-brown in color, a condition aptly described by Weir (66) as "mottled." In the late spring or early summer the apothecia of the fungus appear as large single fructifications or numerous small ones on each side of the midrib on the lower surface of the needle. The epidermis of the needle splits exposing an orange to orange-brown hymenium consisting of asci and paraphyses. When mature, ascospores are released and inoculate the newly developing needles. The majority of the year-old infected needles fall during the spring and summer and by late summer the only evidence of the disease is the sparse foliage.

Disease losses are caused by a considerable reduction in growth, serious defoliation of marketable Christmas trees, and killing of trees. Although infected needles may fall during all seasons of the year, the major period of needle casting in New York plantations was in summer, shortly after the maturation of the ascospores. The best time for an accurate estimate of the damage caused by the Rhabdocline needle cast in New York plantations is in April. During this month, most of the infected needles are still retained, needle mottling is intense and heavily infected needles are completely brown except for the petiole. In May and June, most of the infected year-old needles are already cast. Since infections on new needles are not macroscopically visible during these two months, the only field symptom is the sparse foliage which may be concealed by the new growth in recently infected plantations.

When diseased trees are cut for the Christmas tree market their needles soon become completely brown, dry rapidly, and are cast. Weistaner (68) cites the disastrous effects of the disease on Montana's Christmas tree industry in the epidemic years of 1947 and 1948 when harvested trees were ready for sale.

In western North America, where *R. pseudotsugae* is endemic, sporadic epidemics develop which last for about two years and then decline. The needle cast disease in the northeastern United States and in Europe, where the host and pathogen are out of their natural range, develops into severe epidemics. The disease is now in its eighth year in some of the New York plantations and its damaging effects are rapidly increasing. Long periods of severe *Rhabdocline* epidemics are known for many European plantations of Douglas-fir. Infected trees in New York plantations become about 50 per cent defoliated after three years (Fig. 1) and may be nearly completely defoliated after five years. Weir (66), in Montana, reported that trees that had been attacked for several consecutive seasons were almost completely defoliated and died or "simply existed" without growing. Heavily-infected trees in Scotland were defoliated after five years, and death often occurred after three years of defoliation (70).

R. pseudotsugae, both in this country and in Europe, has been observed on 2-year-old nursery stock and on trees 30 years old or more (5, 66). Most Douglas-fir plantations in New York were established in the late 1930's, hence the effects of the fungus could be determined on only a relatively few age classes.

Both height and diameter growth of trees afflicted with needle cast were reduced in the study plantations. Estimations of growth reductions were complicated by the extreme susceptibility of the trees to late spring frosts. Certain trees in the study group were frost-hardy but susceptible to *R. pseudotsugae* so that estimates of effect of disease on diameter growth were obtained by sectioning (Fig. 2). After the second or third year of heavy infection by *R. pseudotsugae*, the reduced number of needles and living portions of infected needles resulted in a marked slowdown in growth.

Since the initial report of Rhabdocline needle cast disease was made (66) in Montana and Idaho, the known distribution of the disease has increased materially. Martin (33) reported its occurrence in Oregon and British Columbia in 1923. It was first discovered in Scotland in 1922, but was not reported until 1926 by Wilson and Wilson (70). It was believed to have been introduced into Scotland on transplants from the west coast of North America as early as 1914. Disease quarantine laws were advocated by von Tubeuf (60) in Germany and by Van Vloten (62) in Holland but the disease had already become established in these countries. The natural spread

FIG. 1. — Douglas-fir infected three years by *Rhabdocline pseudotsugae*. The foliage is sparse and the tree has no immediate value as a Christmas tree. Frost damage is evident on current year shoots. June 1957.

of the fungus by ascospores, coupled with the wide dissemination of infected nursery stock resulted in a rapid spread of the disease over Germany. In a paper on the advance of *R. pseudotsugae* in Germany, Rohde (45) cited 114 centers of infection by 1934. One center had also been detected in Poland at that time.

During 1938, the Rhabdocline needle cast disease was reported in Douglas-fir plantations in Holland, Switzerland, Denmark, Norway, Sweden, and Czechoslovakia (5). In 1956, Spaulding (54) added Austria, Belgium, France, and Yugoslavia to this list.

In western North America the fungus is ubiquitous but the extent of its damage has been largely undetermined. Sporadic epidemics in Christmas tree plantations have been reported. In addition to the Pacific Northwest and the Inland Empire, *R. pseudotsugae* has been reported in Arizona (15), Colorado (52) and the Northeast. In the Northeast, the fungus has become a serious threat in Christmas tree

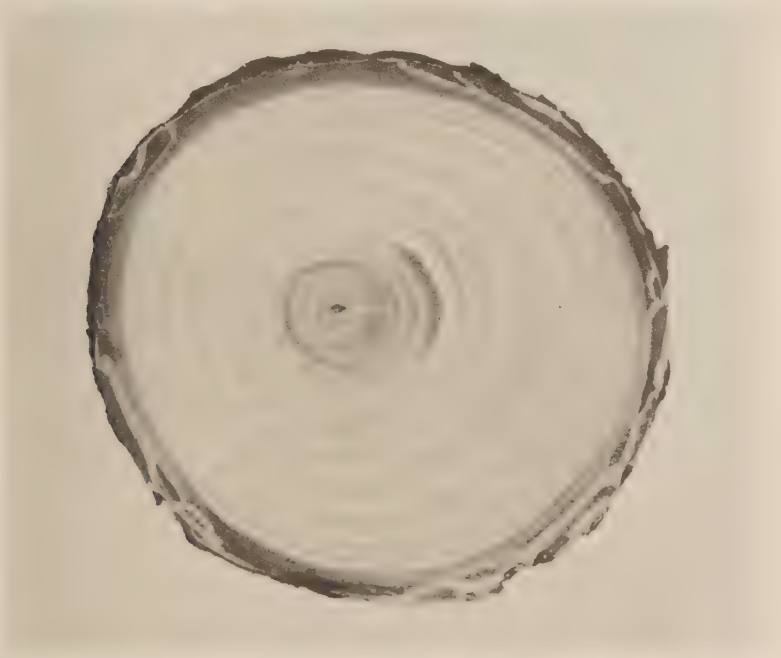


FIG. 2. — Cross-section of a stem of a Douglas-fir infected for five years with *R. pseudotsugae*. The decrease in annual growth, especially during the past two years, is readily visible. July 1957.

plantations. In 1928, Boyce (4) discovered the disease in eastern Massachusetts, and in 1949 Weiss (67) reported it in Massachusetts, New York, and Rhode Island. Stambaugh and Bramble (56) reported the disease in Pennsylvania in 1952.

How the fungus first became established in plantations in New England is unknown (5). Baxter (1) states that the fungus occurs on ornamental Douglas-fir in the Northeast. Boyce (5) believes that the fungus was introduced into the eastern United States from the west coast on transplant nursery stock. Stambaugh and Bramble (56) believe *R. pseudotsugae* in Pennsylvania originated from the shipment of diseased Douglas-fir nursery stock from Colorado to Susquehanna County in the northern part of the state.

The disease is now found in Douglas-fir plantations throughout eastern and central New York. The earliest known record of the disease in the state was that of an outbreak on Long Island in the late 1930's reported by Dr. Donald Welch of Cornell University, Ithaca, New York.

Infected plantations at Camden, Cortland, and Cheningo, New York, all within a 50-mile radius of Syracuse, were used as experimental areas in the present study.

THE NEEDLE CAST DISEASE IN CENTRAL NEW YORK

The development of *Rhabdocline pseudotsugae* has been traced by several authors (29, 46, 66, 70). In general, the various descriptions of the development are in close agreement. However, certain critical periods in the life history of the causal fungus are variable and cannot be predicted without knowledge of local environmental conditions. For example, the time of ascospore maturation and release in a given area cannot be predicted and the duration of spore cast may differ greatly from one area to another. Control measures, to be effective, must be correlated with the proper stage of development of the fungus. The purpose of this phase of the present study was to obtain detailed knowledge of the development of *R. pseudotsugae* in respect to seasonal and environmental conditions existing in central New York.

DEVELOPMENT OF SYMPTOMS

After initial infection of Douglas-fir needles by *Rhabdocline pseudotsugae* in late May or early June, no visible external symptoms of the disease are seen for several months. The early stages are extremely difficult to detect. The first visible evidences are the minute yellow spots on either surface of the needle. These may appear from September to mid-December in New York, depending apparently on weather conditions. Weir (66) first reported such variations in the development in his original descriptions of the disease. In Montana and Idaho the spots were normally observable in December, but during cool, wet summers and autumns such symptoms often appeared two months earlier. Wilson and Wilson (70) reported that yellow spotting of needles occurred in Scotland as early as June. This early date is puzzling since the authors gave late May or early June as the infection period. The early stages of other disorders, such as aphid injury, nutrient deficiency or winter injury, may also cause yellow spotting of needles. Therefore, such symptoms may be mistaken for *R. pseudotsugae*, especially if the disease is known to be present in the area.

Most of the early infections by *R. pseudotsugae* occur near the tips of the needles. The yellow spots enlarge as autumn advances, but

remain sharp in color with their distinct borders abruptly delimiting the dark green of the uninfected portions of the needle. At this early stage, with the aid of a microscope, it is possible to distinguish, but with some difficulty, the colorless hyphae of the parasite within the needles. These hyphae are confined to the area of the lesion and appear to kill the cells of the needle as they advance. Detailed microscopic development is treated subsequently.

In December or January many of the yellow flecks become brown, and with further enlargement coalesce to form large necrotic spots or bands. Other yellow flecks, however, remain light colored throughout the winter and spring. In February or March many of the brown areas are encircled by a yellow-green zone which gradually blends into the healthy dark green areas. The needles in this stage of infection have a typical "mottled" appearance first described by Weir (66) and later by von Tubeuf (61). This is a reliable symptom of the Rhabdocline needle cast disease. However, von Tubeuf (61) states that the mottling develops in summer and remains unchanged over the winter. The author observed mottling in September of 1956 on a few heavily infected trees in the Cheningo plantation. The remainder of the trees in this plantation developed mottling later, even though they appeared to be as heavily infected.

During March and April, the brown necrotic areas continue to enlarge. This may be due to the killing of cells by toxic materials secreted by the overwintering fungus or through desiccation of the cells. Coalescence of the brown areas may become extreme causing the entire needle, with the exception of the petiole, to become brown. On the other hand, individual flecks may remain distinct and separate, resulting later in the production of minute fruiting bodies. The exclusion of the petiole from infection has been cited previously by both Wilson and Wilson (70) and Brown (7) who investigated the cessation of hyphal growth at the juncture of the petiole and lamina.

The brown areas, regardless of their size, deepen in color on the lower surface of the needles. Each brown area is at first slightly sunken and oriented longitudinally in the direction of the bands of stomates. Upon initiation and growth of the apothecia in these sunken areas in April or May, the epidermis expands until it appears as a cushion on the lower surface of the needle. Wilson and Wilson (70) and Boyce (5) state that apothecia occasionally form on the upper surface of needles, but this was not observed in the present study.

FIG. 3. — Lower surface of Douglas-fir needles covered with mature apothecia of the fungus *R. pseudotsugae*. June 1957. (× 7)

MATURATION OF APOTHECIA

In central New York maturation of the apothecia occurred between May 15 and June 1 in 1956 and 1957. In 1956, in the Camden plantation, mature fructifications developed more than a week in advance of those in the Cortland plantations. At apothecial maturity the epidermis, lifted by the growth of the hymenium (FIG. 3), is split lengthwise or raised at the end to partially or completely expose the orange-brown hymenium. In the cool, wet spring of 1956, apothecia in this stage of development were first found May 15 at Camden, and May 21 at Cortland. During the extremely dry April and early May of 1957, one-year-old needles of Douglas-fir infected with *R. pseudotsugae* were fully browned and appeared too dry to persist on the trees. Light but steady rains began May 10, and by May 13 many apothecia were open. In both years apothecia continued to mature and open throughout June and most of July.

Bud opening in 1956 and 1957 was highly variable, depending apparently upon the individual tree and the exposure of the tree.

The importance of genetic factors in bud bursting in Douglas-fir has been recently suggested by Morris, Silen, and Irgens-Moller (38). This would serve to explain the difference between individuals, but variations between buds of the same tree would seem to be due to bud physiological differences or differences in the micro-environment between buds.

Whatever the cause, buds on an individual tree mature at different times and the maturation of apothecia is correlated with the stage of bud development in their immediate vicinity. In 1956, at the Camden plantation, apothecia opened a week in advance of the first bud bursting. However, microscopic examination of these apothecia disclosed morphologically immature asci and ascospores until the buds were opening.

Apothecia on the different trees were highly variable in shape and size and, in general, could be assigned to one of two groups. The first included those fructifications ranging from mere flecks to distinct raised pustules 3 to 4 mm. in length. The spores were cast through small openings at the ends of the apothecia. The second group included those extending longitudinally 10 to 15 mm. along the needle length and opening by means of a slit in the epidermal cover, either at the margin of the apothecium or along an indistinct and jagged median suture. No apparent morphological differences in asci and ascospores could be observed within the two groups. The differences suggest morphological strains of *R. pseudotsugae* in New York plantations. Since the host trees were not genetically uniform this may be a result of host variation; however, the differences were observed on all strains and needle types of the host in the study plots. Wilson and Wilson (70), Peace (40), and Butler and Jones (11) have suggested the possibility of the presence of strains or races of *R. pseudotsugae* in America, on the basis of their ability to infect the various host strains. Peace (40) states that the fungus varies in fructification, spore shape and size, and probably in pathogenicity. Weistaner (68) in his studies of *R. pseudotsugae* in Montana reported that browning of needles varied from small spots to bands across the width of the lamina. However, he stated that the apothecia, asci, and ascospores were the same in each type.

NEEDLE CASTING

The casting of needles may begin during the winter, several months before apothecial maturation. Weir (66) reported that in

Montana infected needles fell at all seasons, while Wilson and Wilson (70) reported that the needles were cast after spores were shed. In the light of the present studies, it appears that both reports are right. Needles are shed at all seasons in New York, but this is a result of weak infections and late infection of lammas shoots. The majority of infected needles are shed during the summer months following ascospore discharge.

Normal needle retention in Douglas-fir was observed by Pease (41) to average from three to nine years. Groom (21), Sargent (50), and Sudworth (57) all reported "about" eight years. Since infection by *R. pseudotsugae* causes needle casting in one year, or two at the most, there is an abrupt decline in photosynthetic capacity and resulting lowered production of wood, even in young trees.

The persistence of infected needles beyond one year has been reported, but the value of such needles to the tree was questioned (61). Brown (7) stated that infected needles are less effective in photosynthesis, since "their producing power is lessened in proportion to the amount of necrotic surface area."

Loss of water by diseased needles has been reported to be instrumental in needle shedding (7, 37, 49). Galloway (18), who studied *Coleosporium* rust of eastern white pine (*Pinus strobus* L.), reported increases in evaporation of as much as one-fifth above the normal rate as the fungus ruptured the needle tissue. This increase resulted in "loss of turgidity and other physiological changes" and shedding of needles. Brown (7) related the loss of water to the premature casting of Rhabdocline-infected needles, but Van Vloten (63) found that neither water loss nor degree of infection influenced needle fall.

In New York, consecutive infections normally resulted in the complete defoliation of twigs. Rohde (48), in comparing the two needle cast diseases, *Adelopus gäumanni* and *Rhabdocline pseudotsugae*, stated that the latter fungus invariably left a certain number of needles uninfected, even in epidemic outbreaks. In New York plantations heavily diseased trees often have their lower branches entirely devoid of needles except for those of the current year. In addition late frosts may kill many buds as the new needles are about to emerge. This occurred sporadically in the spring of 1956, but very heavily in 1957. Hubert (23) remarked that defoliated trees in Montana had a moth-eaten appearance. This was true with even moderately infected trees in New York, especially when the new tips had been frosted (Fig. 4).

FIG. 4. — Moth-eaten appearance of twigs when the needles have been infected by *R. pseudotsugae*. The dark tips of some twigs were the current year's needles, frosted while in the process of emerging from the bud. June 1957.

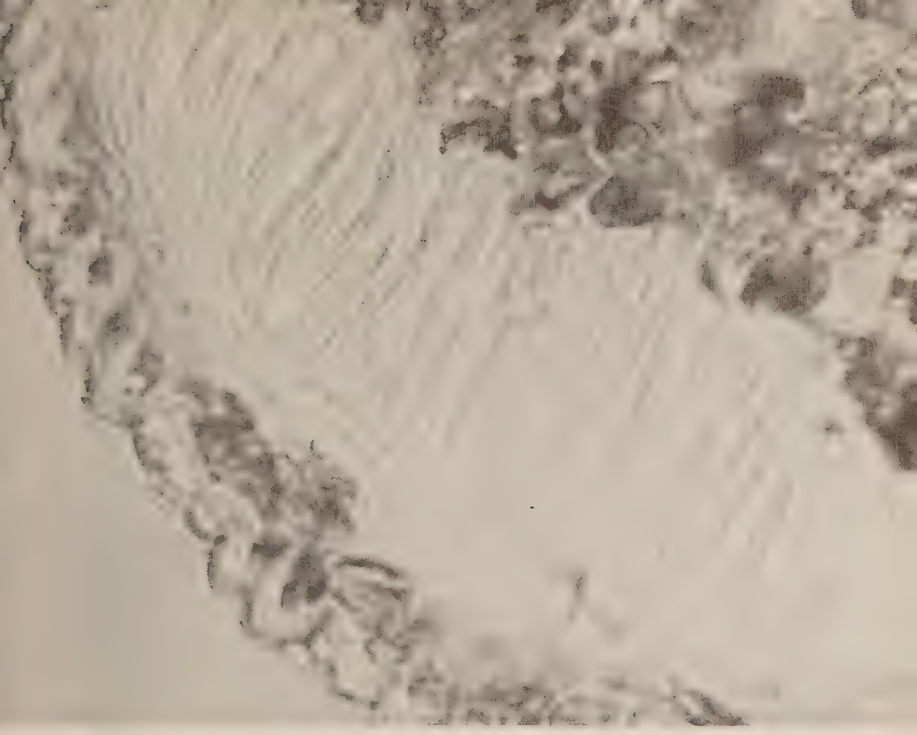


FIG. 5. — Cross-section through an apothecium. The comparatively large size of the asci in relation to the entire fruit body is clearly shown. The epidermis of the needle covering the hymenuim appears in the bottom of the photograph. June 1956. (× 450)

SPORULATION

In 1956 and 1957, very rarely did the hymenium complete its development prior to the initial rupture of the apothecial covering. Since rupturing of the epidermis is the result of the growth and outward development of *R. pseudotsugae,* examination of apothecia at the time of initial splitting of the epidermis invariably revealed a similar stage of morphological maturity (FIG. 5). Ascospores were beginning to form in the older asci near the centers of apothecia. After this stage, which occurred between May 15 and May 25, nearly a week elapsed before asci and ascospores were mature.

Both in 1956 and 1957, the first unicellular ascospores were trapped during the first week of June, at which time the apothecia were considered to be mature. All trapping was by means of vaseline-covered glass slides placed in various places about the infected trees.

The asci were cylindrical to clavate, pointed or abruptly rounded above and very short stalked. The average measurements of 150 mature asci were found to range from 117-138 μ in length to 17-20.5 μ in width. These measurements lie within the limits given by Weir (66), Sydow (58), and Wilson and Wilson (70). The asci were separated by few to many paraphyses which were normally the same length or slightly shorter than the asci. They were hyaline, filiform, and unbranched. Sydow (58) described the paraphyses of Weir's specimens as being forked or simple, whereas Weir (66) described them only as filiform. No branched paraphyses were found among the New York specimens. All paraphyses were consistently less than 3 μ in diameter except for their swollen tips.

Each ascus normally contained eight ascospores. Several authors (23, 66, 68, 70) have stated that the spores are usually obliquely uniseriate and may be irregularly biseriate in the ascus. In this study ascospores were most generally arranged irregularly in two ranks (Fig. 6). The ascospores were commonly unicellular, hyaline and constricted prior to and during expulsion from asci, and had a swollen outer capsule or sheath. This sheath appeared to be very sticky, since large clusters of spores were often found adhering within the apothecium. The spores were shot out of a relatively large pore at the end of the ascus. The pore stained blue with iodine as demonstrated by Weir (66). Von Tubeuf (61) stated that cross-walls often developed before the spores constricted. On the other hand, Sydow (58) stated that the spores were less constricted when mature. Ascospores in the present study were constricted prior to septation (Fig. 6) and remained so in later development (Fig. 7) until just before germination. Most of the ascospores were released in their unicellular stage (Fig. 6), but others became septate prior to release. Wilson and Wilson (70) stated that a few spores do this. In the present study, many ascospores became septate within the ascus (Fig. 7). Unicellular spores, when released, became bicellular and rounded on both ends as previously described (29, 46, 66, 70).

Wilson and Wilson (70), in germination studies with ascospores of *R. pseudotsugae,* stated that in the bicellular spore one cell usually becomes thick-walled and dark while the other remains hyaline. They were able to germinate the ascospores in water and an aborted-looking germ tube was produced from the dark end of the spore.

In this study ascospores were seen to darken at one end, often within the ascus (Fig. 7). Spores released into the apothecial cham-

FIG. 6. — Expulsion of the ascospores from asci showing the common unicellular and hyaline condition of the spores upon release. June 1956. (× 750)

FIG. 7. — Cross-section of asci and paraphyses. The ascospores within asci appear to be quite variable in degree of maturation. The two-celled condition is apparent in many spores with some showing a darkening of one cell. (× 750)

ber in this condition continued to develop. The dark end increased in size, whereas the hyaline end became somewhat distorted (FIG. 8). A large granular spot, approximately one-third to one-fourth the size of the cell sometimes occurred in the dark end. This was only occasionally observed and was not seen after the hyaline cell had again become distended. Ascospores with dark cells and fully distended hyaline cells averaged 18.7 to 20.4 μ by 6.8 to 10.2 μ. These dimensions do not include the sheath, which normally appeared to be quite thin in this advanced stage of maturity. No germ pores were seen, and none are believed to develop in the cell wall prior to extension of the germ tube (FIG. 9).

In 1956 the type of spores just described were not found in apothecia until June 26. Later they were discovered to germinate readily in the apothecium. Such spores were found in an apparently viable state as late as August 2, 1956. In 1957, germinating ascospores were found in apothecia on June 7 and were present as late

FIG. 8. — Partially mature ascospores released from the asci. The hyaline cells are distorted, while the dark cells are round with a light outer rim or halo. (× 950)

as July 15, when the field investigation was terminated. Stambaugh and Bramble (56) stated that germinated ascospores were found in fructifications of *R. pseudotsugae* in Pennsylvania on June 2. They did not describe the general appearance of the spores in relation to Wilson and Wilson's work (70), but stated that a single germ tube developed.

All of the germinating ascospores found within the apothecia, or on the surface of the needles, developed strong lateral germ tubes (FIGS. 10 and 11). Quite often a spore, possibly trapped in some manner, would germinate within the ascus, but even then germination was typically lateral. Occasionally an abnormally enlarged hyaline cell occurred on an otherwise normal ascospore (FIG. 12). Germinated dark cells of such spores always produced dark lateral germ tubes. Rarely could the sheath be seen surrounding the dark thick-walled cell even though it might be obvious around the hyaline cell. After initial growth of about 3 to 5 μ, the germ tube lost its dark color and became hyaline. This occurred in the apothecium,

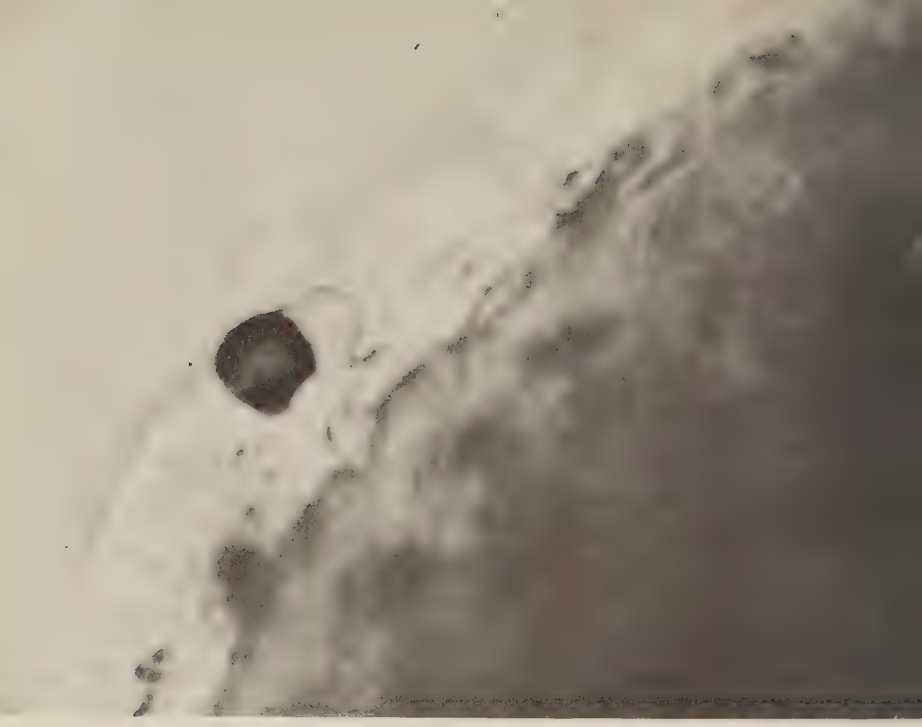


FIG. 9. — Typical early germination stage of the ascospore. The germ tube is evident from the reverse side, looking through the dark cell of the spore. In this spore the hyaline cell remains distended, since the spore was free in the apothecium. July 1956. (× 1100)

but further growth into a branched, septate mycelium characteristic of *R. pseudotsugae* was not observed.

Spore ejection from the ascus was demonstrated by the procedure used by Weir (65) for *Hypoderma deformans* Weir. Portions of moist needles with fresh mature apothecia were placed in the depressions of culture slides. The slides were placed on moist filter paper in petri plate halves, covered, and allowed to dry slowly. After 24 hours spores were observed on and around the needle, up to 1.5 mm. from the apothecial margins. Weir (65) reported that in *H. deformans* the hygroscopic movements of the apothecial lips forced spores from asci. In this study, *Rhabdocline pseudotsugae* spores were actively ejected from apothecia whose epidermal covers had been carefully removed.

Douglas-fir needles, heavily infected by *R. pseudotsugae,* are readily cast after ascospore discharge and, at times, may fall before

FIG. 10. — This ascospore, germinating within the apothecium, clearly shows the typical lateral germ tube shortly after its emergence. (× 2250)

apothecial maturation. Whether ascospore dissemination occurs after needles have fallen has long been a question (28, 61). In the present work, fallen needles with apothecia containing the black germinating cells of the ascospores were found during the period of spore dispersal. When high humidity was maintained beneath the tree, as often occurred where heavy grass or thick mosses were abundant, secondary organisms quickly invaded the entire needle, especially the apothecial cavities. Dried needles were greatly shriveled and no ascospores could be identified among the blackened tissues of the apothecia. Many infected needles lodged among the lower needles of the tree as they fell, but these too dried rapidly and produced no recognizable ascospores.

The abnormally dry spring of 1957, followed by a wet, early summer, resulted in an excessively large number of infected needles remaining on the twigs after the ascospores were cast. The continued high humidity also resulted in the growth of many contaminants. These were largely members of the genera *Penicillium*, *Aspergillus*,

FIG. 11. — A germinating ascospore within the apothecium. The dark germ tube has grown out as a young hyaline hypha. Spores were commonly found in this state as late as August 2. Young asci were still forming below. July 1956. (× 1250)

Alternaria, *Chaetomium*, *Septoria*, and *Stemphylium*. The needles were rotted on the twig, and therefore produced no ascospores of *R. pseudotsugae*.

It appears that spores may form in mature apothecia after infected needles fall, but they are not effective as inoculum due to the rapid deterioration of the needles.

To obtain an accurate appraisal of ascospore dispersal by *R. pseudotsugae*, it was necessary to determine three factors: (1) the time when active liberation of spores first began, (2) the period when spore liberation was at a maximum, and (3) the duration of sporulation. Such information, along with local meteorological data, was collected at the Camden and Cortland plantations.

Temperature and relative humidity readings were taken with Fries hygrothermographs. Three of these were calibrated in the laboratory at 100 per cent relative humidity over a two-week period before use in the field. Papers were changed at weekly intervals at the

FIG. 12. — Abnormal enlargement of the hyaline cell of an ascospore. The halo about the spore and the hyaline tip of the germ tube is formed by the gelatinous sheath. (× 2250)

time spore traps were collected. The instruments were placed at ground level in screened shelters, and located near the infected trees used in the spore-trapping experiments.

Clean, labeled, glass microscope slides were coated with a thin layer of vaseline petroleum jelly. Twelve slides, three per tree, on four infected trees per area, were exposed each week. These slides were attached in vertical and horizontal planes at various heights among infected branches of diseased trees. Such spore traps were maintained at Camden from May 15 to July 31, and at Cortland from May 28 to August 27, in 1956. These slides were placed no higher than four feet above the ground, since the incidence of Rhabdocline needle cast is greater on the lower portions of the tree.

A rectangular cover slip was placed over the vaselined surface of each slide at the end of the weekly exposure period. Replacements were set in the same positions among the branches and exposed slides were taken to the laboratory for examination. The unique dumbbell

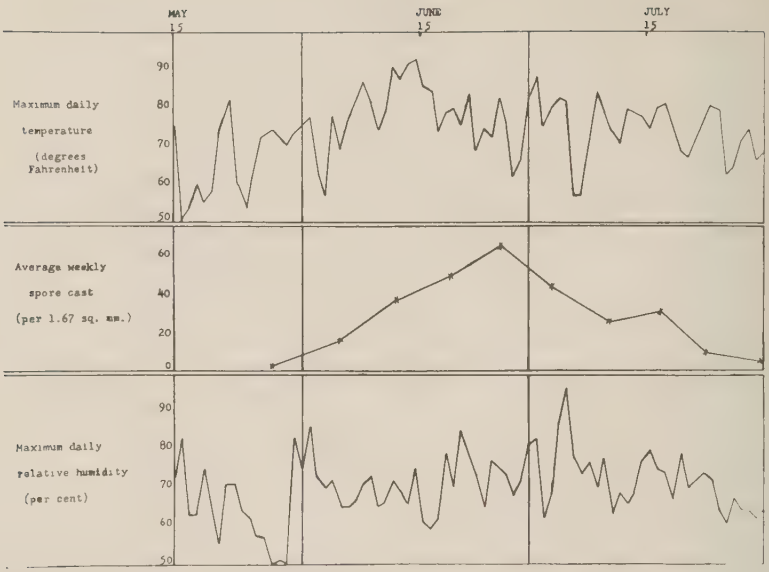


FIG. 13. — Ascospore discharge at the Camden plantation, 1956. Maximum temperature and relative humidity are plotted on a daily basis. The center graph shows the number of ascospores deposited, per 1.67 sq. mm. of slide, per week. Each weekly plot is the average of 12 slides.

shape, comparatively large size, and the cell differences in the ascospores of *R. pseudotsugae* made them easy to identify accurately. An index of spore discharge was based on the number of ascospores of *R. pseudotsugae* counted under low power on a slide area of 1.67 sq. mm.

The results from the spore-dispersal study are presented by graphs relating ascospore discharge to temperature and relative humidity (FIGS. 13 and 14).

Ascospores were first identified on slides from Camden on May 29 (FIG. 13). The gradual warming of the area in late May apparently initiated ascospore discharge, but a sharp increase in relative humidity coincident with a rapid increase in the number of spores trapped, suggests that moisture is more important in sporulation of *R. pseudotsugae* than is temperature. Ascospore dissemination reached a peak at Camden during the week ending June 26, then decreased gradually until the week ending July 17. At that time a renewed

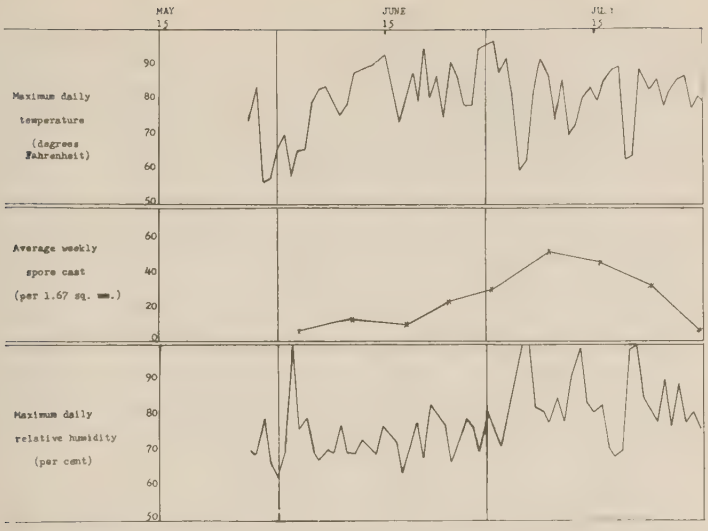


FIG. 14. — Ascospore discharge at the Cortland plantation, 1956. Maximum temperature and relative humidity are plotted on a daily basis. The center graph shows the number of ascospores deposited, per 1.67 sq. mm. of slide, per week. Each weekly plot is the average of 12 slides.

increase in spore liberation occurred. It is interesting to note that this increase occurred during a week of high relative humidity.

The first ascospores were found at Cortland on June 4 (FIG. 14). The initiation of spore casting and the peak of sporulation were coincident with periods of high relative humidity. The peak sporulation was reached at Cortland on July 9, nearly two weeks later than at Camden.

There was little relationship between time of ascospore discharge and temperature. The temperature at both areas fluctuated continuously, with no extremes in either direction lasting longer than two days. The average maximum temperature for June and July at Cortland was 80°F., while that at Camden was 76°F.

INFECTION

The parasitic nature of *Rhabdocline pseudotsugae* and its ability to attack the needles of the most vigorous trees as frequently as those of diseased or suppressed trees is well known (29, 66, 70). The exact nature of infection, however, is still not clear.

FIG. 15. — Ascospores adhering to the surface of a young needle. The hyaline ends of the spores have partially disintegrated. The black cell of the ascospore on the left shows a very young germ tube apparently about to penetrate through the cuticle. (× 800)

Studies on pathogenicity, mode of infection, and physiology of *R. pseudotsugae* would be facilitated if it were possible to grow the fungus on artificial media. Subsequent investigation of varietal reaction of host trees and of the suggested heterothallism of the pathogen (70) would also be possible. All attempts to culture *R. pseudotsugae* failed, thus tending to substantiate Van Vloten's (63) suggestion that the fungus is an obligate parasite. However, if *Rhabdogloeum hypophyllum* is the imperfect stage of *Rhabdocline pseudotsugae*, the obligate nature of the latter might still be questioned, since the former has been successfully cultured (15, 68) and conidia were produced.

To obtain more information on the method and site of needle penetration, fresh apothecia were scraped from infected needles and placed in sterile distilled water. After two hours this inoculum was applied to the tender needles of newly expanded shoots of potted trees in the greenhouse. After 6, 12, and 24 hours, needles were coated with egg white (applied by means of an atomizer) in an attempt to hold spores onto the surface of the needle during subsequent preparation for sectioning. Young needles were excised from the twig, killed and fixed in formalin-acetic acid-alcohol, dehydrated using Johansen's (24) tertiary butyl-ethyl alcohol series, and em-

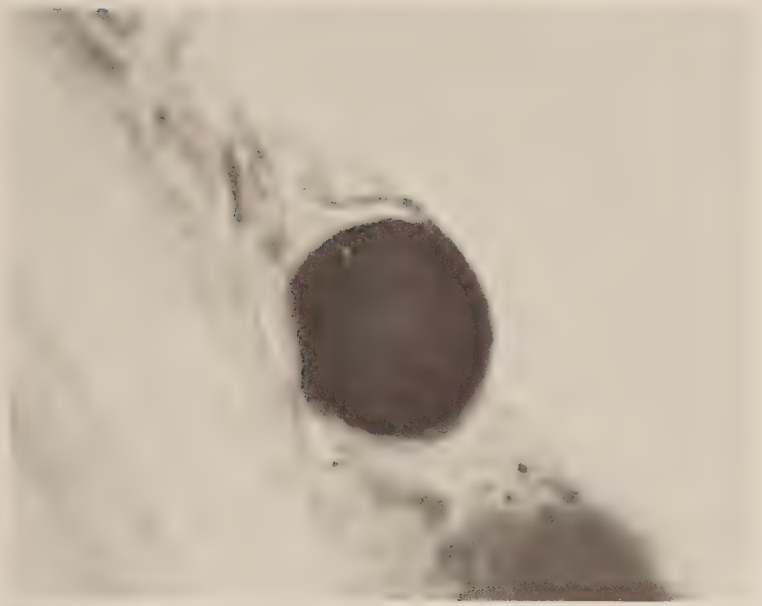


FIG. 16. — Closeup of an ascospore on a needle immediately prior to germination. The light cell has collapsed and expanded over the surface of the needle. (× 2500)

bedded in paraffin. Young, naturally-infected tips were also collected from the field and treated in a similar manner. Longitudinal sections of needles from these tips were prepared and various stages of spore germination and subsequent infection were studied.

Infection was initiated by mature ascospores as the needles unfolded from the buds. The spores were extremely sticky when moist and became attached to the needle surface (FIG. 15). After contact, the hyaline end of a spore disintegrated and its gelatinous sheath spread outward, thus cementing the dark end firmly to the needle. A small protuberance from the black cell (FIG. 16) was the first indication of germination, unless germination had occurred previously in the fungus apothecium as described. The young, dark-colored germ tube elongated and soon after penetration of the epidermis became hyaline and septate. Penetration was directly through the cuticle as observed by Van Vloten (63). Hubert (23) suggested that the fungus might penetrate the needles through stomatal openings, but entrance through stomata was not observed in this study. An infection peg passed through the outer wall of an epidermal cell

or at the juncture of two epidermal cells. If the spore had germinated in the apothecium, and later came in contact with a young needle, the existing germ tube oriented itself perpendicularly to the cuticle and then penetrated the needle. No more than a single germ tube per spore was observed. The continued branching, septation, and production of the intracellular mycelium is discussed later.

RATE OF SPREAD

The Douglas-fir plantations in New York, because of their small size, were suitable for only limited studies on the rate of spread of *R. pseudotsugae*. Determination of the rate of spread made by a disease in years past can only be made if infection on different age needles can be dated. Two common methods for determining the age of needles are by (1) counting bud scars which mark the boundaries of annual shoot growth, and (2) making free-hand sections through the twig at the base of a given needle for counting annual growth rings. Both methods give rise to errors if lammas shoots develop. If lammas growth occurred late in a given year, a twig might show a new annual ring consisting of large tracheids adjacent to typical summer wood tracheids. Two growth rings would, therefore, be formed in that year. If lammas growth occurred early, the new large tracheids would adjoin spring wood tracheids and only one growth ring would be formed. However, two bud scars could be counted for that year.

Since the needles of lammas branches were often infected, they were also cast and could not be used to fix the age of infection. The infection stage of *R. pseudotsugae* was often used to differentiate between normal spring growth and the later lammas shoots by means of differences in the stage of disease development.

After a relatively large sample of trees were studied a distinct infection pattern developed. In every case the spread of the diseased trees formed a somewhat fan-shaped pattern in the direction of the prevailing wind. In the plantations studied the prevailing wind was toward the East. In all but the Camden plantation, the extension of the disease was up the slope from the primary infections.

In each of the infected plantations, the primary infection occurred on a relatively few trees, ranging from one to four. The infected trees, if more than one, were usually close together. At Cortland, however, two primary infection centers, approximately 200 feet apart, were located. The disease had spread over the intervening distance in two years.

Although the spread of the disease at Cheningo approximated the general pattern described above, a block of trimmed trees within the plantation appeared to cause a slight deviation in direction of spread. These trees were on the windward side and downslope of the plantation. Except for a fringe of infected needles on trimmed trees bordering the untrimmed heavily infected trees, no evidence of *R. pseudotsugae* infections was found. It is not known whether the prevailing crosswind prevented viable spores from reaching these trees or whether control was accomplished by the increased aeration with subsequent rapid drying of otherwise susceptible needles. The primary infection on four trees on the extreme western side of the stand resulted in nearly 100 per cent infection of trees up to 200 yards to the East in three years.

The plantations studied varied in size from approximately four to ten acres. In all cases the disease had spread over the plantations within three years after initial infection. Many trees which initially escaped infection later became diseased. These apparently were less susceptible to infection by *R. pseudotsugae*.

On the basis of the present study, the rate of spread of *R. pseudotsugae* in central New York plantations might be expected to vary from about 100 to 250 feet per year in the direction of the prevailing wind. It will be somewhat less in other directions, depending upon local topography, wind exposure and moisture conditions. While the figures given for the spread per year are approximate, they were obtained during a period of extremes in climatic conditions such as may be expected to occur in central New York. The extremely hot, dry summer of 1955 was followed by an excessively cool, moist spring and summer in 1956. By far the greatest spread of the disease occurred in the 1956 season. The increase in intensity of needle cast infection during cool wet seasons has been reported by Weir (65, 66) and Boyce (5).

Plantations infected for four successive years had approximately 55 to 75 per cent of the trees infected. In the Camden plantation nearly 50 per cent of the diseased trees were estimated to be more than 40 per cent defoliated.

Prolonged studies of the spread of Rhabdocline needle cast in the Northeast are prohibited due to the limited size of Douglas-fir plantings, and the rapidity with which the disease envelops such small stands.

LAMMAS GROWTH

The occurrence of lammas growth is a phenomenon quite commonly observed in trees. Many hardwoods, notably species of beech (*Fagus* L.) and oak (*Quercus* L.), develop vigorous secondary shoots which often exceed the length of the spring shoots (10). Among coniferous species European larch (*Larix decidua* Mill.) has been reported (9) to form late shoots, and the author has observed them on Scotch pine (*Pinus sylvestris* L.), red pine (*Pinus resinosa* Ait.) and eastern white pine (*Pinus strobus* L.). Büsgen and Münch (10) state that lammas shoots occur more commonly on younger trees, and cite the "green" Douglas-fir as an example. Lammas branches are associated with the formulation of a terminal bud in the middle of the summer. These buds undergo a brief period of rest, a month or slightly less in Douglas-fir. The part of the annual shoot formed after the pause in such cases appears as new growth to which the name "Lammas shoot" has been given in view of the approximate date[1] of its appearance.

The relationship of lammas growth to the Rhabdocline needle cast disease has been overlooked due to the supposedly short sporulation period of the fungus. Liese (29) in a study on the biology of *R. pseudotsugae*, considered lammas shoots but believed that they developed too late to be infected. The overlapping of the period of spore discharge with lammas shoot expansion suggested the probable importance of this phenomenon in the development and spread of the disease.

Lammas shoots are usually very vigorous. In Douglas-fir they sometimes equal or surpass the spring growth, but often fail to ripen or harden-off. This may result in frost kill of the new terminals and resultant poor form of the tree in succeeding years. Such damage was common in the Camden area, and as a result many trees developed poor forms unsuited for Christmas trees even if the effects of *R. pseudotsugae* were not considered. Another factor promoting the disfigurement of trees which produce late shoots is the susceptibility of such shoots to insect attack.

All branches of a tree do not exhibit lammas growth. Certain trees in a plantation, and certain plantations in an area, vary in late shoot development. In the Cortland area a relatively few lammas branches developed in 1956, whereas in the Cheningo area, only 5

(1) Lammas day was a bread festival celebrated in the past by Roman Catholics on August 1.

miles away, lammas production was abundant.

The exact cause of lammas shoot formation is unknown. According to Büsgen and Münch (10) several factors seem to be involved including nutrition, the accumulation of food reserves and an abnormal water supply. Brown (8), in a study of forking in hardwoods in southern England, found late branching largely dependent upon degree of shading. He observed less lammas growth on strongly shaded plants. Above-normal summer rainfall, such as the prolonged midsummer rains of 1956, appeared to promote lammas growth. In the present study trees, with and without lammas shoots, were found in the field with their branches intermingled. Therefore, it appears that inherited tendencies as well as environmental factors are important in relation to late summer shoot extension.

Lammas shoots occurred on Douglas-fir in the Camden, Cortland, and Cheningo plantations in 1955, 1956, and 1957. Two distinct stages of *R. pseudotsugae* infections were observed in the Cortland area in November 1955. On lammas needles initial yellow spotting was just becoming apparent, while on spring needles intense mottling had developed and the needles were somewhat brown and dry.

Once lammas infection was established, and the 1955 and 1956 seasons indicate this to be frequent, the fungus developed on a schedule somewhat behind that of the spring infection. Even though late infections occurred, many of them appeared to be very weak, as if the spores were less pathogenic or, late shoots possessed more resistance to the pathogen than did spring shoots. These apparently weaker infections needed more time to develop and produce mature fruit bodies. Lammas needles with incipient apothecia were observed as late as the second winter. Many needles dried and fell prior to apothecial maturation, but some remained to produce inoculum the following spring.

During the spring and summer of 1956 the time of bud development was observed at the three study areas. Details are given for the Camden area only, since bud development appeared about one week later in a similar pattern in the other two areas. On June 8, less than a month after spring buds burst at Camden, terminal buds were observed on some of the branches. On June 15, many of these terminal buds were one-quarter inch long while the laterals were nearly one-eighth inch long. At this time the majority of the 1955 spring-infected needles had been cast but the greater portion of the 1955 lammas needles remained and were still developing apo-

thecia. Eleven days later (June 26), lammas shoots were beginning to develop and buds were still opening as late as July 23 on some trees. An important fact here is that a second flush of needles developed while the apothecia were still producing ascospores. Ascospores were found within apothecia at Camden as late as August 2.

In 1957, buds on spring shoots were mature on July 8. Some were opening at this date, but they were few and scattered. Adventitious buds were developing at this time also, both along the branches previously defoliated by the needle cast disease, and around the frosted terminal buds. By July 14, very few buds were breaking, but on microscopic examination of apothecia it was found that asci and ascospores were still forming on 1956 lammas shoots.

The environmental conditions which are conducive to the formation of late shoots are apparently similar to those which are necessary for the development of the disease on the previously infected spring needles. This was true during the 1956 season. The prolonged summer and fall precipitation advanced the fungal development to such an extent that by late November, needles which were normally only faintly spotted, were red-brown and often exhibited the erumpent cushion form of the young apothecia. These symptoms are not normally seen until late March or early April of the following spring.

The overlapping life cycles of the spring and late shoot infections and the effects of heavy precipitation in accelerating the development of the disease suggest reasons for the varying reports on the symptomatology of the disease. The periods of initial yellow spotting and later merging of these spots were difficult to limit in point of time. Certain trees, with lammas shoots, were found with both yellow flecks and red-brown blotches on needles throughout the summer, whereas other trees, with or without lammas growth, showed no visible evidences of infection until late December.

HISTOPATHOLOGICAL STUDIES

While some work has been done on the development of *Rhabdocline pseudotsugae* within infected needles (63), little reference is made to the early stages of the fungus at, and immediately following, the time of infection. Adequate reports of the incipient stages of apothecial formation have not been published nor is detailed description of the development of the hymenium available. The purpose of this phase of the study was to investigate the histopathology of the Rhabdocline needle cast disease to provide the answers to some of these problems.

Diseased needles were collected, cut into short sections, and immediately killed and fixed in formalin-acetic acid-alcohol (24). Immediately upon reaching the laboratory, air was evacuated from the tissue with a water aspirator. The material was stored in formalin-acetic acid-alcohol for a minimum of 48 hours and then dehydrated in a tertiary butyl-ethyl alcohol series (24). A mixture of equal parts of tertiary butyl alcohol and paraffin oil was used to effect a gradual transfer from the alcohol to paraffin. Fisher Tissuemat (M.P. 56-58.5° C.) was used for both infiltration and final embedding. After softening in a mixture of equal parts of glacial acetic acid and 60 per cent alcohol for 36 hours, serial sections were cut, 10 μ thick, and mounted on slides with Sass adhesive III (51).

A stain combination of 1 per cent chlorazol black E in 70 per cent ethyl alcohol and 1 per cent water-soluble solutions of safranine O and crystal violet was used for all staining. The first two stains delineated the needle structures, whereas the crystal violet differentiated the fungus from the host tissue.

NORMAL NEEDLE ANATOMY

Douglas-fir needles are differentiated into dermal, mesophyll and vascular tissues. A relatively thick protective cutin covers the outermost or epidermal layer of cells. The remaining one to three layers of cells in this region consist of thick-walled hypodermal cells. These long, fiber-like cells are lignified and circumscribe the needle, with the exception of the area containing the bands of stomata on the lower surface.

The mesophyll consists of large, irregular parenchyma cells

which contain the chloroplasts and thus constitute the site of photosynthetic activity within the needle. The mesophyll region is nearly equally divided between these thin-walled cells and air spaces. Resin canals are present near the lower surface of the needle on each side of the midrib.

The endodermis separates the mesophyll from the central pericycle and fibrovascular bundle. The endodermal cells are large, thickwalled and somewhat ovoid-elongate. Since the pericycle and vascular bundle are not injured in any way by the fungus they are not mentioned further.

DISEASED NEEDLE ANATOMY

After infection of a needle by *R. pseudotsugae* the ascospores remained attached to the surface of the needle. The dispersion of ascospores by rain water and moist winds apparently tended to concentrate the relatively heavy ascospores on the upper surfaces. Then too the smoother upper sides of needles, as opposed to the jagged lower sides, seemed to afford a better surface for attachment of spores.

The black germ tube of the ascospore, and the hyaline extension of the germ tube, penetrated directly through the cuticle regardless of its thickness. No hyphae were evident in the stomata of needles. Relatively thin hyphae penetrated the juncture between epidermal cells and also grew between hypodermal cells rather than passing through them. Upon reaching the mesophyll, the hyphae became enlarged, septate and almost entirely intracellular. These hyphae were not found until early September of 1956. By October the hyphae had become branched, were globular in shape and appeared to be filled with oil (Fig. 17). The hyphae varied from 3 to 5 μ in diameter. Liese (29) stated that septation of such hyphae were scarcely visible, but in the present study crosswalls were seen as soon as hyphae were visible.

Throughout the winter months, the hyphae penetrated further into the needle, killed more cells, and thereby increased the size of the visible lesion on the surface of the needle. Epidermal cells of infected needles appeared to remain unchanged and did not differ morphologically, nor react differently to stain, than did those of healthy needles. The hyphae were largely of the coarse intracellular type, but as the winter progressed, a few thin intercellular hyphae were found throughout the needle. At times these penetrated the

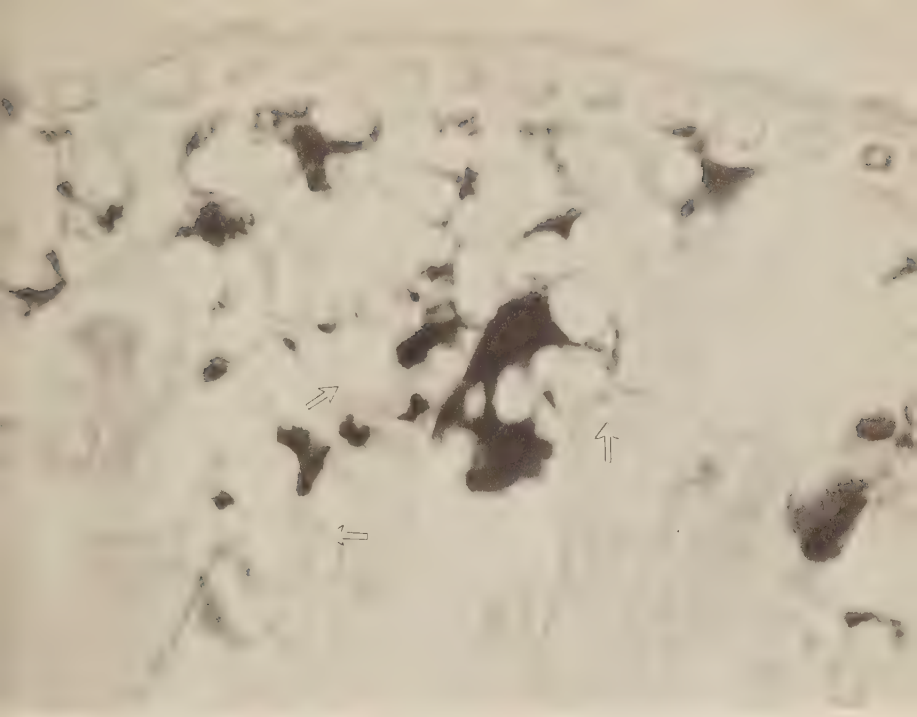


FIG. 17. — In October, 1956, globular oily hyphae (arrows) of *Rhabdocline pseudotsugae* were readily apparent in the mesophyll cells. Many of these cells were partially collapsed and their contents were blackened. (× 1200)

epithelial cells and entered the resin canals. Brown (7) and Van Vloten (63) stated that hyphae were never observed within the endodermis. In this study large accumulations of hyphae were commonly observed around the endodermal ring (FIG. 18). Some strands appeared to penetrate the outer wall of the endodermal cells, but this could not be determined with certainty. The thin-walled passage cells, though few in number, were definitely infected. No hyphae were observed within either the transfusion tissue or fibrovascular bundle.

By early April, the fine intercellular hyphae in the cells of the upper mesophyll were turgid (FIG. 19), were relatively thick-walled and more regular in diameter than during the winter months. The cells of the upper portion of the needle were mostly deteriorated. The chlorenchyma adjacent to the lower epidermis was often intact though filled with the globular type hyphae (FIG. 20). By late April

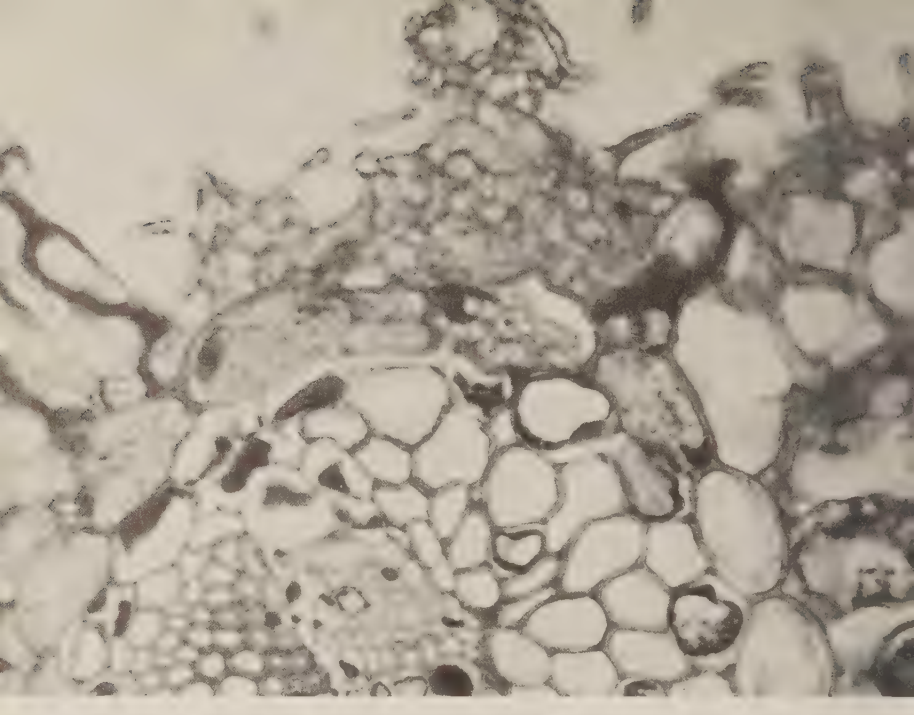


FIG. 18. — Concentration of hyphae of *Rhabdocline pseudotsugae* closely appressed against the endodermal wall. The presence of protoplasmic material in the endodermal cells was obvious, but it could not be determined whether this was a manifestation of the host or of the pathogen. (× 800)

or early May, host cells in this area rapidly broke down and the hyphae multiplied in number. Thus a closely-appressed mass of hyphal tissue formed, usually directly beneath the stomata (FIG. 21).

In mid-May, strands of hyphae toward the epidermis became parallelly aligned to form a distinct hymenium. The hyphae toward the interior of the needle became less appressed, large cavities developed and the hymenium became virtually suspended within the needle. Asci and paraphyses formed at the tips of the hyphae in the hymenium. Needles collected on June 4, 1956 revealed the first morphologically mature ascospores. Little change, other than subsequent deterioration of the mesophyll appeared within the needles throughout the remaining summer months. Two-celled ascospores with germinated black cells were found in apothecia collected on August 2, 1956. Whereas the hymenium was shriveled and stained very heavily, a few ascospores were still visible within the asci.

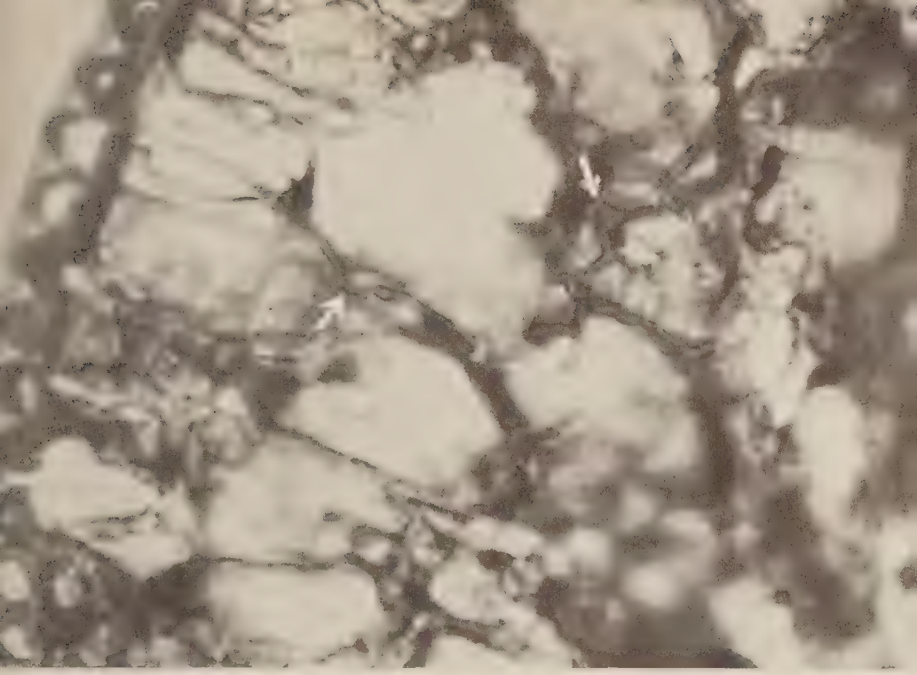
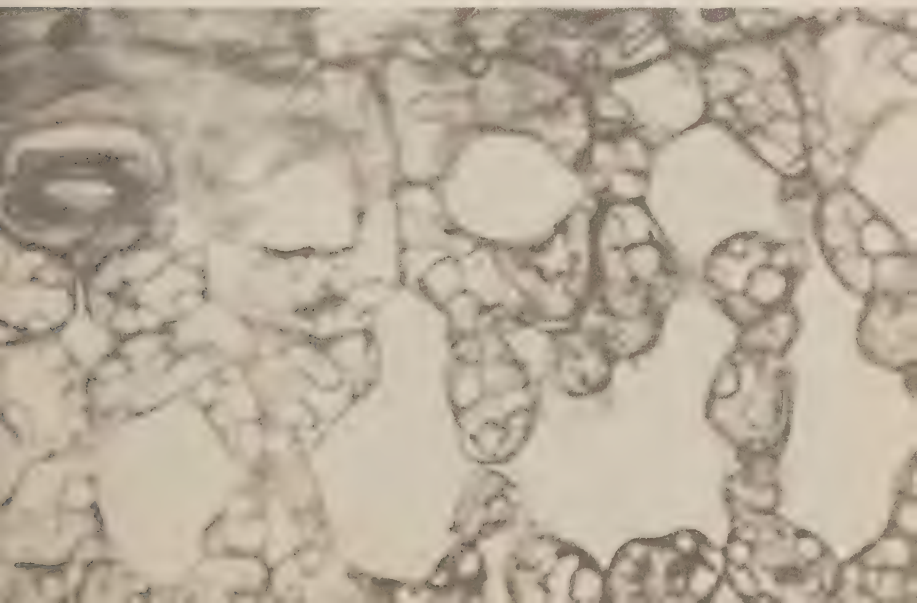


FIG. 19. — Thin intercellular hyphae (arrows) near the outer margin of the needle. By early April they were fully extended and quite regular in diameter. (× 800)

FIG. 20. — Longitudinal view of the mesophyll just below the lower epidermis. A stomate and its guard cells are visible at the upper left. Globular septate hyphae are abundant in the chlorenchyma. April 1956. (× 1200)



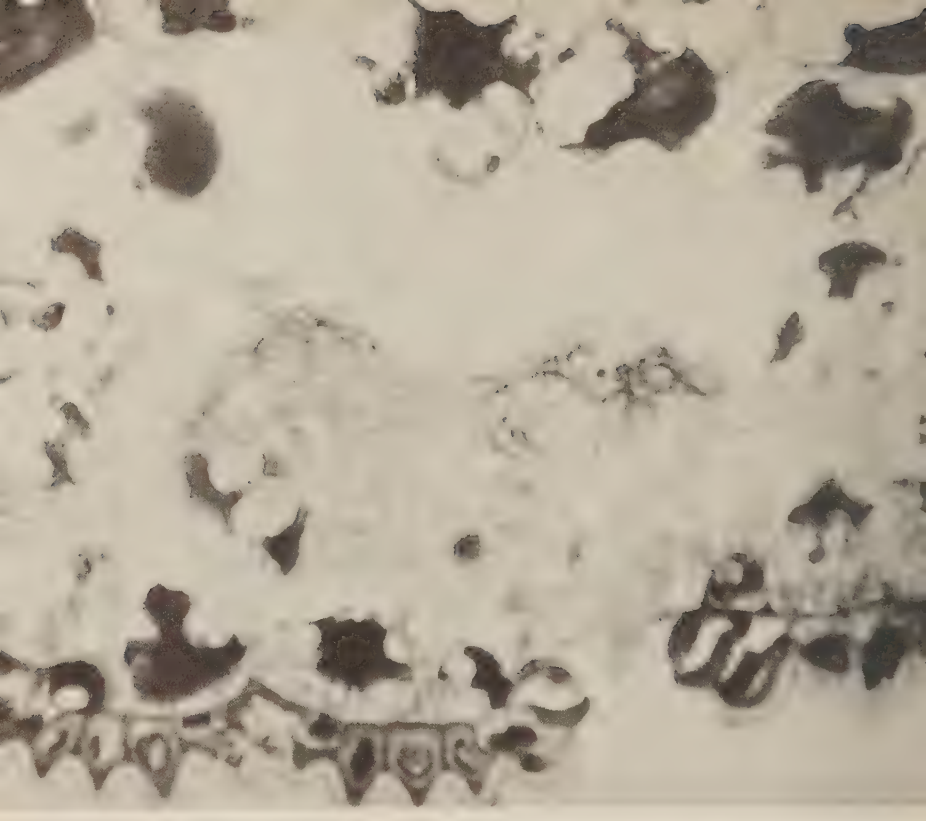


FIG. 21. — Cross-section of a Douglas-fir needle showing a mass of hyphae concentrated under a stomate. Few hyphal strands connect the mass with the mycelium in the remainder of the needle. (× 1000)

CONTROL STUDIES

Since the initiation of the current studies on Rhabdocline needle cast, spray control plots at various plantations of the Northeast have been maintained. It was thought that the use of spray programs to provide acceptable disease-free trees would be economically justifiable because of the high stumpage value of Douglas-fir for Christmas trees. It seemed obvious that such a program should be initiated by a search of known fungicides in the hope of finding one or more that would control *R. pseudotsugae*.

In 1956 test plots were established at the Camden and Cortland plantations in New York. Forty-five trees, with varying degrees of infection, were selected in each plantation and given numbers. These numbers were then randomly assigned to five different treatments, using Snedecor's (53) random numbers tables. Ten trees were assigned at each plantation to each of four different spray materials: Bordeaux mixture, Lime-sufur, Ferbam, and Captan. Five trees were sprayed with flour and Dreft (used as sticker and spreader respectively) in water, as a check on the possible toxicity of this mixture. The remainder of the infected trees in each plantation served as untreated controls.

In 1958 and 1959 additional plots were established in Douglas-fir plantations on the University of Vermont forest at Jericho, Vermont, and on a private estate at Woodstock, Vermont. Trees in both of these plantations were heavily infected with both *Rhabdocline pseudotsugae* and *Adelopus gäumanni* Rohde. Fifteen trees were sprayed with one of three compounds: Cyprex, Actidione BR, or Actidione S. Thus a total of forty-five trees was sprayed at each location. All toxicants with their common names and concentrations are given in Table 1.

The fungicides were applied either by means of 5-gallon copper knapsack-type or 3-gallon galvanized garden-type spray tanks. Only the lower 6-foot portion of trees were sprayed except for trees 6 to 7 feet in total height wherein the entire tree was sprayed.

The first spray was applied at Camden on May 22 and at Cortland on May 28, in 1956. The sprays were then applied at weekly intervals through the end of June. Two later applications, one on July 14 and the other on July 23, were made at Camden when it was observed

TABLE 1

SPRAY MATERIALS USED IN THE CONTROL OF RHABDOCLINE PSEUDOTSUGAE [a]

Toxicant	Common name [b]	Concentration of toxicant per cent
Copper sulfate	Bordeaux mixture	1.0
Calcium polysulphides	Lime-sulfur	0.7
Ferricdimethyl-dithio-carbamate	Ferbam	0.3
N-trichloromethylmercapto-4-cyclohexene-1-2-dicarboximide	Captan	0.2
Dodecylguanidine acetate	Cyprex	0.2
Cycloheximide (Beta-2- (3, 5-dimethyl-2-oxocyclohexyl)-2-hydroxy-ethyl-glutarimide	Actidione BR	0.001 0.0005
Cycloheximide (Beta-2- (3, 5-dimethyl-2-oxocyclohexyl)-2-hydroxy-ethyl-glutarimide semi-carbazone	Actidione S	0.0005

[a] A mixture of Dreft, flour, and water was used as a control.

[b] The Bordeaux mixture was a 4-4-50 formula as recommended by Boyce (5). The Lime-sulfur was manufactured by Goulard and Olena, Inc.; Ferbam by E. I. duPont Co.; Captan by the California Spray Chemical Corporation; Cyprex by American Cyanamid Co.; and the Cycloheximide derivatives by the Upjohn Co.

that lammas shoots were developing while ascospores were still being produced. Thus a total of five spray applications were made on each tree at Cortland and seven at Camden. The 1958 spray programs at Jericho and Woodstock, Vermont, were restricted to two applications of each trial fungicide; the first application being made as the Douglas-fir buds were beginning to open and the second 10 days later.

To obtain a measure of the efficacy of the various spray materials used, observations were made in April of the year following treatment. A visual inspection method was used to compare the appearance of the sprayed foliage with that of unsprayed trees. The relative greenness and retention of the past year's needles was the best criterion for assaying the effectiveness of the different fungicides. Of importance, but secondarily so, was the relative number and developmental stage of the fruiting bodies of *R. pseudotsugae*. To facilitate

reporting the results of the control studies, it has seemed best to analyze each fungicidal material separately as to its effectiveness in controlling the disease.

Needles of trees sprayed with Bordeaux mixture have constantly been found to be heavily infected and appear to be much drier than unsprayed needles during the spring of the year following spray applications. There were certain indications that the fungicide had actually stimulated fungus development on some needles. Infection by *R. pseudotsugae* was especially heavy on the lower branches of Bordeaux-sprayed trees. Needles of lammas shoots appeared to be as heavily infected as those of spring shoots on some trees. Adventitious buds which developed along the bare twigs of these trees produced needles which also became infected. Generally, the foliage of Bordeaux-sprayed trees appeared to become excessively thin. Indeed, many of the spring-infected needles were cast by March and April of the following year, before apothecial maturation.

The consistent failure of Bordeaux mixture to give control was unexpected since it has been recommended specifically for the control of the disease by several authors (1, 5, 13, 16, 66, 68). Fischer (16) in Germany, and Weistaner (68) in Montana, reported control with a 4-4-50 concentration similar to that used in the present studies. The subsequent drying of Bordeaux-sprayed infected needles was not mentioned by either investigator. The lack of drying of sprayed young and old non-infected needles leads to the conclusion that the spray stimulated *R. pseudotsugae* in some manner, thus indirectly causing the more rapid and complete dessication of needles through greater fungal activity. The action by Bordeaux throughout the course of the present study has certainly been inconsistent with existing reports of its use against *R. pseudotsugae* and needs further investigation.

Lime-sulfur gave nearly 100 per cent control at Camden and Cortland, New York, in 1956. A few apothecia developed on scattered basal branches but probably as a result of inadequate spray coverage. Control was excellent on early spring needles as well as those of lammas shoots. The lower sprayed portion of such trees made a sharp contrast with the heavily infected unsprayed upper part of the tree. There were no signs of burning of foliage by the use of a seven-tenths per cent water solution of Lime-sulfur. Weistaner (68) used Lime-sulfur on Rhabdocline needle cast in Montana, but it provided no control. He theorized that his solution might

well have been too weak to effect control. A concentration of 1 to 40 rather than the recommended summer strength of 1 part concentrated solution to 50 parts of water was used throughout the course of the Lime-sulfur trials in the present study.

Indications are that *R. pseudotsugae* may be controlled using Lime-sulfur sprays, if the first application is made at the proper time in relation to bud burst in spring, and repeated at 7- to 10-day intervals through the initial 2 to 3 weeks of lammas shoot development. One can immediately foresee the economic and physical problems inherent in such an intensive program. Limiting the applications to two in number with the first at the time of bud burst and the second 10 days later failed to control the disease in the Vermont studies of 1958. These latter tests were not conclusive, however, since a second severe needle cast disease, caused by the fungus *Adelopus gäumanni* Rohde, was present on the test trees and can be expected to have altered the results of the tests in some way.

Control of the Rhabdocline needle cast with Ferbam in New York plantations varied from poor to very good. Infection on the lammas branch needles was controlled in all cases. At Camden, of 10 trees sprayed with Ferbam in 1956, 2 showed complete control of *R. pseudotsugae* on spring needles, and on 7 other trees control was good except for the north and northeast sides of the crown. It is conceivable that some type of peculiar temperature-moisture relationship existed on the surface of the northernmost needles causing dilution and subsequent ineffectiveness of the Ferbam. Needles on these sides were lightly to moderately infected. The tenth tree was heavily infected throughout. Needles of the spring shoots at Cortland had light to moderate infection and by comparison with unsprayed needles it was questionable whether any control had been achieved. North and northeast exposures of Ferbam-sprayed trees were not infected at Cortland as they were at Camden.

Two treatments with Ferbam in the Vermont studies, applied the same days as the Lime-sulfur trials reported above, failed to give any control of *R. pseudotsugae* in 1958.

Limited control was obtained with Captan on a single tree with the exception of the north side which was severely diseased. This condition of three-sided control was commonly observed on Ferbam-sprayed trees at Camden. The year-old infected needles which had been sprayed with Captan were very dry and brittle, even more so than Bordeaux-sprayed needles. Older uninfected needles showed

no apparent ill effects from the spray. Although fungus stimulation by the spray may have caused the excessive injury, it is more likely that the new foliage was injured by the Captan. Some Captan-sprayed trees produced very little new growth in the spring of 1957, one year after spraying, but a definite relationship could not be drawn. It was not felt that the Captan was effective enough to be continued and hence was not used in the Vermont studies.

Cyprex was used only in the Vermont phases of the control studies in plantations where both the Rhabdocline and Adelopus needle cast diseases were serious. The first applications were made in 1958 on June 2 and 3 due to the late development of Douglas-fir buds in the Jericho and Woodstock areas. A second application after 10 days completed the test. The foliage of these trees appeared to be a little greener than that of the surrounding unsprayed foliage for the summer and fall months. By late October, however, this difference had vanished and infection by *R. pseudotsugae* appeared to develop as strongly as on the unsprayed trees. By April of 1959, not one of the 30 trees (15 each at Jericho and Woodstock) yielded evidence of any degree of *Rhabdocline* control.

The cycloheximide derivatives, Actidione BR and Actidione S were used in the Vermont test plots only. The use of Actidione BR at the recommended concentration of 0.001 per cent proved far too toxic to the tender young foliage at the time of bud break and thus resulted in complete and irrevocable burning of the 1958 foliage. The concentration was reduced to 0.0005 per cent for the 1959 trials, resulting in only a slight burn to some of the new growth. The effectiveness of Actidione BR as a control for Rhabdocline needle cast has not been evaluated to date.

The use of Actidione S analog of cycloheximide for foliar application has followed the unhappy results of the 1958 tests with Actidione BR. Since the S analog was applied in late May and early June of 1959, the life cycle of *R. pseudotsugae* is but partially complete and the effectiveness of the spray cannot be ascertained as yet. Inasmuch as the cycloheximides are eradicant sprays, there might possibly be more hope for this essentially different approach to control than for a continuation of protectant spray trials.

Relatively few other spray materials have been used or even recommended by others to combat *R. pseudotsugae*. Weistaner (68) used Phygon (2, 3-dichlor-1, 4-napthequinone) at two teaspoons per gallon of water but with no success. Various concentrations and

formulations of Bordeaux have been used, some with partial control as mentioned previously.

Control work in the past has not been entirely confined to spraying. Many European investigators advocated legislation to prohibit the importation of Douglas-fir nursery trees, in an effort to exclude *R. pseudotsugae* from established plantations (11, 60, 62).

Some of the earliest control recommendations were based on felling of diseased trees during autumn and winter and burning infected branches before spring (27, 42). Geyr (19) maintained that the best method was by developing resistant or immune varieties. As this view became accepted many reports of varietal differences in resistance were published (6, 31, 35, 36, 39, 44, 46, 47, 54, 73).

The selection of resistant trees on warm dry sites has been advocated by Merkle (34) for the control of Adelopus needle cast disease, and might well apply for the control of *R. pseudotsugae*. Wide divergences in relative resistance of the various strains of Douglas-fir to the disease are readily seen in the plantations of the Northeast (Fig. 22). The intermingling of branches of healthy and diseased trees has been continually observed throughout the duration of this study. It seems apparent that selection of individuals for needle cast resistance, with coordinate programs of propagation, whether by means of cuttings, grafts, or both, will be necessary if Douglas-fir is to be grown successfully in the region. Of interest here is the increasing concern shown, by Douglas-fir plantation managers in the Pacific Northwest, toward the dual threat posed by severe and continuing outbreaks of *R. pseudotsugae* and *A. gäumanni* in their natural and original locale. Such a combined menace in the natural range of the Douglas-fir adds an imperativeness to the question of selection for disease resistance not presented by even the most favorable economic inducements in the Northeast and Europe.

FIG. 22. — Individual differences in susceptibility of *Rhabdocline pseudotsugae*. Such an intermeshing of healthy and heavily infected trees was encountered on all the infected plantations studied. June 1957.

SUMMARY AND CONCLUSIONS

A study of the Douglas-fir needle cast disease caused by *Rhabdocline pseudotsugae* Syd. was carried on from July 1955 through the summer of 1959. Continued attack by this fungus on young Douglas-fir seriously affects the quality and quantity of trees produced for the Christmas tree market.

The development of the Rhabdocline needle cast disease in New York was studied intensively and found to be intermediate between the development as described for western United States and for Europe. The first evidences of the disease were found from September to December depending upon weather conditions. The typical mottling described by previous workers was observed throughout the winter.

Apothecia were of two distinct types recognizable on the basis of their size and manner of opening and were observed only on the lower surfaces of Douglas-fir needles. Development of apothecia was outlined and average measurements of asci, paraphyses, and ascospores given. Ascospores germinated readily in the apothecium and produced a dark black lateral germ tube. No imperfect stage of the fungus was found.

Infected needles were cast throughout the year, but most were shed shortly after dissemination of the ascospores.

Spore dispersal was of longer duration than previously suggested. Apparently-viable germinating spores were trapped two months after the last Douglas-fir spring buds had burst. From the standpoint of control this means that spray applications must be extended further into the summer than would otherwise be required. However, the time of opening of spring buds and of lammas buds are the critical periods since the tender new needles are apparently very susceptible to infection. High humidity favored dispersal of ascospores as well as the incidence of infection, but no relationship could be found between spore dispersal and temperature.

Infection occurred with equal frequency on vigorous as well as on suppressed trees. After a spore settled on a needle surface, the hyaline end deteriorated and spread outward to cement the ascospore to the needle. Penetration was through the cuticle of either

surface and never through stomata as suggested by Hubert (23). The present study indicated that all trees, except for a few apparently resistant individuals, can be completely infected if conditions are favorable for the fungus. The disease was found to have a short-distance spread of from 100 to 250 feet per year. Spores are disseminated by moisture-laden winds as was suggested by Weir (66).

The role of lammas shoots, in relation to the Rhabdocline needle cast disease was investigated. They have been reported to develop too late to become infected (29) but were commonly infected in New York. The extension of the spore dispersal period for *R. pseudotsugae* answers four pertinent questions on the activities of the causal fungus. One, the apparent duplicity in appearance of symptoms is explained by the parallel, but distinctly separate, development of two groups of infected needles. The spring needles were heavily infected, whereas needles of late or lammas shoots produced typically weak, slow-developing infections. Two, the reported (5) retention of infected needles for two years before apothecial development is found to be directly related to the fact that although many infected lammas needles develop minute apothecia by the following spring, most of these infections are so weak, and develop so slowly that two years are needed for the fungus to fruit. Three, lammas infection, which may have failed to develop at the end of one year, might easily be mistaken for infection of two-year-old needles, especially if it was uncertain whether lammas growth had occurred. Lastly, if lammas branches were not infected, some of them would retain needles entirely free from the disease. In central New York, even though lammas branching occurred during the summers of 1955, 1956, and 1957, the twigs of infected trees were entirely defoliated.

The formation of lammas shoots makes it difficult to develop an adequate spray program. The late development of apothecia on lammas needles could maintain or even increase the disease in an area where successful control was achieved on spring needles.

Histopathological studies substantiate the previous suggestion that penetration by *R. pseudotsugae* may occur on either surface of the needle. There is much evidence to suggest that the upper surface is by far the most commonly penetrated. The development of two kinds of hyphae within the needle is in agreement with the findings of previous workers (7, 29, 63). Concentrations of hyphae were closely appressed against the endodermal cells but failed to penetrate beyond. Arrangement of hyphae to form the hymenium was

described as was the deterioration of the mesophyll in the vicinity of the developing apothecium.

Control of the fungus by cutting out infected trees is out of the question and adequate economical control by the use of fungicide appears almost as impossible. Further trials using the two most successful compounds, Lime-sulfur and Ferbam, might be warranted but it is seriously doubted if the Christmas tree producers would be willing to stand the expense and labor necessary to apply sufficient applications at the proper times. A much more promising approach appears to be that of developing resistant individuals. Wide divergences in relative resistance have been observed throughout the Northeast and it is apparent that selection and propagation of these individuals by means of cuttings or grafts will be necessary if Douglas-fir is to be grown successfully in the region.

Apart from future work involving the fungus-host relationship, more conclusive studies are needed on the economics of spraying. With the continued development of new spray materials it is possible that an effective, less expensive material might be found. As stated above, wide divergences in relative resistance to *R. pseudotsugae* are evident and the selection and propagation of trees showing such resistance seems to be the most obvious and practical approach for future studies. The search for and propagation of, resistant trees will involve a long-term costly program. With the Rhabdocline needle cast disease, however, the basic problem may be not merely a matter of increasing yield or improving a product, as is so often the case, but rather the saving of an entire Douglas-fir Christmas tree industry in the Northeast.

LITERATURE CITED

1. Baxter, D. V. 1952. Pathology in forest practice. John Wiley and Sons, Inc., New York.
2. Boyce, J. S. 1926. Pacific Northwest conifers and their diseases in Great Britain. Timberman 27: 170-176.
3. ———. 1927. Observations on forest pathology in Great Britain and Denmark. Phytopathology 17: 1-18.
4. ———. 1929. The future of forest parasites in the United States. Jour. Forestry 27: 138-142.
5. ———. 1948. Forest pathology. McGraw-Hill Book Co., Inc., New York.
6. ———. 1954. Forest plantation protection against diseases and insect pests. F. A. O. Forestry Development Paper, No. 3.
7. Brown, A. B. 1930. Observations on leaf fall in the Douglas fir when infected with *Rhabdocline pseudotsugae* Syd. Ann. Appl. Biol. 17: 745-754.
8. Brown, J. M. B. 1953. Forking in young beech. Forestry Comn. Forest Res. Rpt., London. 1951-1952 (59).
9. Burger, H. 1944. Johannistriebe der Larche. Ztschr. f. das Gesam. Forstw. 76: 10-12.
10. Büsgen, M., and E. Münch. 1929. The structure and life of forest trees. John Wiley & Sons, Inc., New York.
11. Butler, E. J., and S. G. Jones. 1955. Plant pathology. Macmillan and Co., Ltd., London.
12. Clements, F. E., and C. L. Shear. 1931. The genera of fungi. H. W. Wilson Co., New York.
13. Day, W. R. 1927. A leaf-cast disease of the Douglas fir due to *Rhabdocline pseudotsugae* Syd. Quart. Jour. Forestry 21: 193-199.
14. Ellis, D. E. 1939. Conifer diseases hitherto unreported from the Southwest. Plant Dis. Rptr. 23:341.

15. ———., and L. S. Gill. 1945. A new *Rhabdogloeum* associated with *Rhabdocline pseudotsugae* in the Southwest. Mycologia 37: 326-332.

16. Fischer, H. 1938. Die Douglasienschütte. Blumen u. Pflanzenbau ver mit Gartenwelt 42: 331-332.

17. Frothingham, E. H. 1909. Douglas fir — a study of the Pacific Coast and Rocky Mountain forms. U.S. Forest Service, Cir. No. 150.

18. Galloway, B. T. 1896. A rust and leaf casting of pine needles. Bot. Gaz. 22: 433-453.

19. Geyr, H. v. 1932. Rhabdocline — Bekämpfung. Forstarchiv. 8: 326-327.

20. ———. 1932. Nochmals: Rhabdocline. Forstarchiv. 8: 241-245.

21. Groom, P. 1909. Trees and their life histories. London.

22. Harlow, W. M. and E. S. Harrar. 1950. Textbook of Dendrology. McGraw-Hill Book Co., Inc., New York.

23. Hubert, E. E. 1931. An outline of forest pathology. John Wiley and Sons, Inc., New York.

24. Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill Book Co., Inc., New York.

25. Kanzow, H. 1937. Die Douglasie Aufstellung einer Ertragstafel auf Grund der Ergebnisse der preussischen Probeflächen und Auswertung von Provenienzversuchen. Ztschr. f. Forst u. Jagdw. 69: 65-93; 113-139; 241-271.

26. Liese, J. 1931. Zur Rhabdocline krankheit der Douglasie. Forstarchiv. 7: 341-346.

27. ———. 1932. Bemerkungen zu vorstehenden Ausfuhrüngen. Forstarchiv. 8: 245-247.

28. ———. 1932. Die Douglasiennadelschütte und die Möglichkeit ihrer Bekämpfung. Deut. Dendrol. Gesell. Mitt. 44: 294-304.

29. ———. 1932. Zur Biologie der Douglasiennadelschütte. Ztschr. f. Forst u. Jagdw. 64: 680-693.

30. ———. 1933. Weitere Mitteilungen über die Douglasiennadelschütte. Deut. Dendrol. Gesell. Mitt. 45: 268-270.

31. ————. 1936. Die Douglasienrassen und ihre Anfälligkeit gegenüber der Douglasiennadelschütte *(Rhabdocline pseudotsugae)*. Deut. Dendrol. Gesell. Mitt. 48: 259-263.

32. Little, E. L., Jr. 1953. Check list of native and naturalized trees of the United States (including Alaska). Agriculture Handbook No. 41, U.S. Department of Agriculture, Forest Service, Washington, D. C.

33. Martin, G. H. 1925. Diseases of forest and shade trees, ornamental and miscellaneous plants in the United States in 1923. Plant Dis. Rptr. Supp. 37: 349-446.

34. Merkle, R. 1951. Über die Douglasien — Vorkommen und die Ausbreitung der Adelopus — Nadelschütte in Württemberg-Hohenzollern. Forst u. Jagdztg. 122: 161-191.

35. Meyer, H. 1951. Die Verbreitung der Douglasiennadelschütten. Zum Stand der Krankheitserforschung. Forstarchiv. 22: 5-11.

36. ————. 1954. Rhabdoclinebefall an Douglasien verschiedener Provenienz. Forst u. Holzw. 9: 180-182.

37. Miyake, K. 1902. On the starch of evergreen leaves and its relation to photosynthesis during Winter. Bot. Gaz. 33: 321-340.

38. Morris, W. G., R. R. Silen, and H. Irgens-Moller. 1957. Consistency of bud bursting in Douglas-fir. Jour. Forestry 55: 208-210.

39. Murray, J. S. 1953. Recent research on forest pathology in Holland. Quart. Jour. Forestry 47: 41-47.

40. Peace, T. R. 1939. Forest pathology in North America. Forestry 13: 36-45.

41. Pease, V. A. 1917. Duration of leaves in evergreens. Amer. Jour. Bot. 4: 145-160.

42. Plettenberg, V. 1931. Rhabdocline pseudotsugae. Forstwiss. Centbl. 53: 772. Rev. Appl. Mycol. 11: 142.

43. Rohde, T. 1932. Die Folgen des Rhabdocline Befalls in deutschen Douglasien beständen. Forstarchiv. 8: 317-326.

44. Rohde, T. 1932. Das Vordringen der Rhabdocline Schütte in Deutschland. Die Folgen des Rhabdocline – Befalls in deutschen Douglasienbeständen, Welche Douglasien sind in Deutschland durch Rhabdocline gefährdet? Forstarchiv. 8: 247-249; 317-326; 389-392.

45. ———. 1934. Kann man *Rhabdocline pseudotsugae* durch Ausheib vertilgen? Forstarchiv. 10: 121-123.

46. ———. 1934. Zur Biologie der Douglasienschütte. Ztschr. f. Forst u. Jagdw. 66: 151-156.

47. ———. 1936. Schüttegefährdung verschiedener Douglasien-'Herkünfte'. Ztschr. f. Forst u. Jagdw. 68: 610-616.

48. ———. 1938. Ueber den Krankheits verlauf bei der Schweizer Douglasien schütte. Ztschr. f. Pflanzenkrank. 48: 49-57.

49. Sachs, J. 1875. Textbook of Botany (English Translation). Clarendon Press, Oxford.

50. Sargent, C. S. 1902. Silva of North America, Houghton-Mifflin Co., New York.

51. Sass, J. E. 1940. Elements of botanical microtechnique. McGraw-Hill Book Co., Inc., New York.

52. Shope, P. F. 1943. Some Ascomycetous foliage diseases of Colorado conifers. Univ. Colo. Studies, Sect. D. 2: 31-43.

53. Snedecor, G. W. 1956. Statistical methods. The Iowa State College Press, Ames, Iowa.

54. Spaulding, P. 1956. Diseases of North American forest trees planted abroad. Agriculture Handbook No. 100, U.S. Dept. of Agriculture, Forest Service, Washington, D. C.

55. Stambaugh, W. J. 1952. A study of the needle cast diseases of conifers in Pennsylvania. The Pennsylvania State College, University Park, Pennsylvania. Master's thesis.

56. ———., and W. C. Bramble. 1952. The occurrence of Rhabdocline needle cast of Douglas fir in Pennsylvania. Plant Dis. Rptr. 36: 292-293.

57. Sudworth, G. B. 1908. Forest trees of the Pacific slope. U.S. Dept. of Agriculture, Forest Service, Washington, D. C.

58. Sydow, H. and F. Petrak. 1922. Ein Beitrag zur Kenntnis der Pilzflora Nordamerikas, insbesondere der nordwestlichen Staaten. Ann. Mycol. 20: 178-218.

59. Terrier, C. A. 1942. Über das Auftreten der Rhabdocline — Douglasienschütte in der Schweiz. Schweiz. Ztschr. f. Forstw. 93: 14-18.

60. Tubeuf, C. v. 1930. Die Gefahr der Rhabdocline für die Douglasie in Deutschland und ihre Bekämpfung. Ztschr. f. Pflanzenkrank. 40: 390-392.

61. ———. 1932. Rhabdocline — Erkrankung an der Douglasie und ihre Bekämpfung. Ztschr. f. Pflanzenkrank. 42: 417-426.

62. Van Vloten, H. 1930. Aantasting van *Pseudotsugae taxifolia* Britton (Douglasspar) Boschbouwk. Tijdschr. 3: 283-298. Rev. Appl. Mycol. 10: 140.

63. Van Vloten, H. 1932. *Rhabdocline pseudotsugae* Sydow, oorzaak eener ziekle van Douglas spar (German resumé). [Wageningen] Landbouwhoogesch., Proefschrift. Rev. Appl. Mycol. 12: 63.

64. ———. 1935. Wie wird die Rhabdocline-schütte der Douglasien verbreitet? Forst. Wochenschr. Silva. 23: 17-18.

65. Weir, J. R. 1916. *Hypoderma deformans,* an undescribed needle fungus of western yellow pine. Jour. Agr. Res. 6: .277-288.

66. ———. 1917. A needle blight of Douglas fir. Jour. Agr. Res. 10: 99-103.

67. Weiss, F. 1949. The revised check list of diseases of economic plants of the United States. Plant Dis. Rptr. 26: 58.

68. Weistaner, D. A. 1955. Some studies on *Rhabdocline pseudotsugae* Syd. in western Montana. Montana State University, Missoula, Montana, Master's thesis.

69. Wilson, M. 1927. Defoliation of the Douglas fir. Gard. Chron. No. 4506, May 7.

70. ———., and Mary J. F. Wilson. 1926. *Rhabdocline pseudotsugae* Syd.: A new disease of the Douglas fir in Scotland. Roy. Scot. Arbor. Soc. Trans. 40: 37-40.

71. Wollenweber, H. W. and H. Richter. 1932. Die Douglasienschütte und ihre Erreger, *Rhabdocline pseudotsugae* Syd. Blumen u. Pflanzenbau 47: 167-168.

72. Zycha, H. 1948. Ist die Douglasie gefährdet? Forst u. Holz. 3: 231-232.

73. ———. 1951. Pflanzenschutz-Tagung der Biologischen Bundesanstalt Braunschweig in Gosler, 11-14 Oktober 1950. Biol. ZentAnst. Mitt., Berlin 70: 140 pp. Rev. Appl. Mycol. 31: 534.